The Study and Application of Underwater Decomposition from an Entomological Perspective for the Purpose of Post-mortem Interval Estimation

Helen Louise McGonigal

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ABSTRACT

Introduction

While decomposition and insect succession on land are well understood, much less is known about these processes in aquatic environments. The aim of this thesis is to present a series of studies designed to investigate these processes in the South of England, beginning with a questionnaire and semi-structured interviews with various practitioners to assess the need for this type of research to take place. This is followed by a series of field studies recording and comparing decomposition and insect & invertebrate colonisation across different aquatic environments.

Overall these studies provide new knowledge about insect succession in freshwater in the South of England, and some steps have been made towards making ocean-based research more accessible for small laboratories. Additionally, research suggests that the main requirements of Senior Investigating Officers (SIOs) is for forensic entomology data to be presented in a way that is easily understandable and usable in casework (unpublished data, Chapter 4), and these studies represent the first steps towards being able to provide data that meets these requirements.

Forensic Entomology and Underwater Death Investigation: A Review of its Utilisation and Potential

To assess the need for an investigation into aquatic decomposition, a questionnaire was designed to establish the current scope and utilisation of forensic entomology in aquatic death scene investigations, and was distributed to forensic practitioners and other professionals involved in underwater death investigations. Following this, a focus group was conducted with Senior Investigating Officers and was designed to explore experiences of professionals with more managerial experience. The outcomes suggest that there is considerable scope for use of entomology at aquatic death investigations, but that it is not being used to its full potential. Practitioners welcomed future research and agreed that there is a need for better awareness of entomology, but emphasised the need for better engagement between the forensic scientist and investigator.

Influence of Two Enclosed Water Types on Entomological Species Colonisation in Portsmouth, UK

A pilot study was undertaken to collect some preliminary data and begin to develop a methodology for the in-depth studies. Here, two rabbit *Oryctolagus cuniculus* Linnaeus (Lagomorpha: Leporidae) carcasses were decomposed in lidded plastic boxes in an urban garden environment. One box was filled with water and sediment obtained from a local stream and the other was filled with sea water and sand collected from the Solent (Portsmouth, Hampshire, UK). Entomological samples were collected from both carcasses until they were both fully disarticulated (151 days) and the decompositional changes were also monitored. A catalogue of insect specimens is provided. Some initial guidance for collection of entomological specimens from remains found in small enclosed aquatic environments is also proposed.

A Preliminary Investigation of Faunal Colonisation of Remains in Open Water

Here, three different approaches for investigating decomposition and insect succession in an ocean environment were tested with a view to providing a methodology appropriate for use by small labs without access to expensive and highly specialised equipment. Method 1 used a modified crayfish pot to enclose a piglet carcass and incorporated a GoPro™ camera to monitor decomposition and marine fauna feeding behaviour using timelapse photography. Method 2 made use of a whelk pot to house the carcass and improve the rate of trapping feeding fauna. Finally, method 3 took a combined approach using a lobster pot to both house the piglet carcass and trap feeding fauna, as well as using timelapse photography to monitor decomposition and feeding behaviour. Method 3 was found to yield the largest amount of usable data of the three methods tested, primarily showing an abundance of shore crabs.

A Checklist of Arthropods Associated with Piglet Carcasses Decomposing in a Freshwater Pond Environment in Southeast England

In this experiment, four piglet *Sus scrofa domesticus* Linnaeus (Artiodactyla: Suidae) carcasses were allowed to decompose naturally in a man-made freshwater pond located in a woodland area near Wickham, Hampshire, UK. Throughout the

decomposition period, insect specimens were collected and the changes in decomposition state were recorded until the carcasses were fully skeletonised. As with the pilot study, a catalogue of colonising insect species is provided.

Effects of Environmental Temperature on Oviposition Behaviour in Three Blow Flies Species of Forensic Importance

Various factors are known to affect blow fly behaviour with respect to oviposition of which temperature is the most significant factor. These variables apply equally in aquatic environments, however much less is known about the processes of decomposition and invertebrate colonisation in these environments. Here, the oviposition behaviour of three species of forensically important blow fly (*Calliphora vicina*, *Calliphora vomitoria* and *Lucilia sericata,*) was studied under controlled laboratory conditions over a range of temperatures (10 to 40°C). Lower temperature thresholds for oviposition of 16°C and 17.5°C were established for *C. vomitoria* and *L. sericata* respectively, whilst *C. vicina* continued to lay eggs at 10°C. *C. vomitoria* and *L. sericata* both continued to lay eggs at 40°C, whilst the highest temperature at which oviposition occurred in *C. vicina* was 35°C. While this study was not conducted in an aquatic environment, it nonetheless provides important background information for understanding insect colonisation of remains in water, where temperatures may be lower than on land.

Contents

Declaration

Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

Word count: 64, 437

List of Tables and Figures

Abbreviations

CSI	Crime Scene Investigator
DEFRA	Department for Environment, Food & Rural Affairs
DTT	Development threshold temperature
DVI	Disaster Victim Identification
EAFE	European Association for Forensic Entomology
EU	European Union
IGC	Insect Growth Chamber
GAM	Generalised Additive Model
GPR	Ground Penetrating Radar
mPMI	Minimum Post-mortem Interval[1]
NGO	Non-Governmental Organisation
PMI	Post-mortem Interval
PMI_{min}	Minimum Post-mortem Interval
Pre-CP	Pre-Colonisation Period
PMSI	Post-mortem Submersion Interval
ROSPA	Royal Society for the Prevention of Accidents
ROV	Remotely Operated Vehicle
SAR	Search and Rescue
SIO	Senior Investigating Officer
SOP	Standard Operating Procedure
UN	United Nations
UNHCR	United Nations High Commissioner for Refugees (The UN Refugee Agency)
VENUS	Victoria's Experimental Network Underseas
WHO	World Health Organisation
WSART	Water Search and Rescue Team

[1] The abbreviation PMI_{min} is used throughout this thesis with the exception of chapter 3 (*Effects of environmental temperature on oviposition behavior in three blow fly species of forensic importance)* where mPMI is used instead, in accordance with the original published version of the article.

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Dissemination

Publications

Ody, H., Smith, P., & Brown, K. (2017) A Comparison of Decomposition of Rabbit Carcasses in Two Enclosed Water Systems to Inform Death Investigations in the South of England. *Portsmouth Postgraduate Review.* 6 [Online] Available at: https://sites.google.com/a/port.ac.uk/portsmouth-postgraduate-review/issues/six-15-june-2017

Conference Presentations

5th-8th June 2019 "A Preliminary Investigation of Faunal Colonisation of Remains in Open Water", conference poster presented in absentia at the European Association for Forensic Entomology 16th Annual Meeting 2019, Bordeaux, France

21st-25th Aug 2017 " The Study And Application of Underwater Decomposition From An
Entomological Perspective For The Purpose Of Post-Mortem Interval Estimation", conference poster presented in absentia at the 21st Triennial Meeting of the International Association of Forensic Sciences, Toronto, Canada

15th June 2017 "Underwater Decomposition and Forensic Entomology: Application to
Missing Persons Investigations", paper presented at the 3rd International Conference on Missing Children and Adults, Abertay University, Dundee

7th June 2017 "The Study And Application of Underwater Decomposition From An Entomological Perspective For The Purpose Of Post-Mortem Interval Estimation", conference poster presented at the European Association for Forensic Entomology 14th Annual Meeting 2017, Treviso, Italy

25th May 2016 "The Study and Application of Underwater Decomposition", conference
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18th May 2016 "The Study and Application of Underwater Decomposition", conference
poster presented at the Faculty of Health and Social Sciences Postgraduate Conference,
Portsmouth, England

1. Personal Introduction

When I first began designing my research project, I knew from the beginning that I wanted to focus on aquatic environments. I had become interested in decomposition and insect colonisation in water when I was studying for my Masters degree, which explored blow fly oviposition behaviour under different temperature conditions. However, due to the many and varied difficulties with undertaking this kind of project – such as lack of access to water in the area, I was unable to continue with it at that time. Starting a PhD seemed like the perfect opportunity to revisit the topic as I would be able to take advantage of better access to equipment, a larger timeframe to complete the study, and more wide-ranging industry links to draw on expertise from other professionals both within and outside the University.

Drawing on existing literature, I designed a study to compare decomposition and insect succession on remains in a freshwater and seawater environment. This seemed to me to be both a knowledge gap, with very few studies being published on this topic, and an important area in which to improve understanding for police investigations due to the propensity for human remains to be found in water. Portsmouth seemed like the ideal choice to undertake this research as it is a coastal city with documented cases of human remains being found in the Solent (the body of water separating mainland England and the Isle of Wight (Solent Protection Society, 2019)) and other water courses nearby. Working with the Marine Biology department, I was able to secure a location to undertake the seawater part of the study, using the research raft in Langstone Harbour. Through Dr Brown I also managed to gain access to some private land to undertake the freshwater study and a land study for comparison, something which has not previously been conducted in the UK.

Throughout the study, I wanted to collect data which would benefit Crime Scene Investigators (CSIs) and other police personnel in situations where human remains were recovered from water. Apart from filling a knowledge gap, one aspect of

forensic investigation that has always appealed to me is the capacity to help people, in this case through providing additional information which may provide closure for families of the deceased in aquatic death situations. I felt this benefit would come through providing additional information about any "target" species to look for and which species are associated with different stages of decomposition, thereby adding value to the existing processes for investigating deaths in, as well as recovery of remains from, bodies of water. This information is generally lacking worldwide, with the exception of a few studies from select areas, but in particular there is very little from the United Kingdom. Despite this lack of information, it is common to find human remains in water and many examples of cases from the UK can be drawn on ranging from numerous incidences of students drowning in nearby bodies of water after a night out, to the locally infamous case of David Guy, whose dismembered body washed up on Southsea beach in 2012, wrapped in bin liners and a curtain.

In addition, I hoped to provide insight into useful and robust methodologies for conducting this type of study on a small scale – that is to say without the need for large and expensive equipment or specialised personnel (e.g. divers). Clearly the need for such equipment is problematic as it precludes smaller or lesser funded laboratories from contributing to the knowledge base and it would benefit the field for both small and large scale projects, from across the world, to provide data. In this regard I feel I have achieved my aims and although this was a relatively short project compared to some, it underscores the importance of having this type of data available.

Based on observations made as I worked on the project I found other avenues to explore which complement and add additional value to the research. Although I have not had the opportunity to work on these other studies alongside completing my PhD, I have been able to incorporate these ideas into future research plans for myself as well as ideas for student projects. Expanding beyond investigating insect succession, these ideas include researching survival of blow fly eggs in water (see

Appendix 4) and an investigation of damage to submerged remains caused by feeding crustaceans. These research projects are exciting and multidisciplinary, reflecting the recovery of a cadaver from water and requiring knowledge of entomology, anthropology, oceanography, marine biology, limnology and taphonomy, and therefore will require further study on my part as well as collaboration from colleagues and other researchers with wide ranging expertise.

On a professional level, there were a number of benefits for me in undertaking the study. I would be able to improve on existing skills such as insect identification as well as expanding my knowledge and experience of decomposition and forensic entomology in aquatic settings. I would also be able to focus on professional networking and engaging with the worldwide community of forensic entomologists to develop working relationships with researchers in other laboratories. More personally, I was excited to have the opportunity to pursue research on a topic that had interested me for a while but which I had lacked the means to undertake. It would also allow me to expand beyond my current horizons in terms of working in a different lab, and working on a much larger scale project than I had done previously thereby building confidence and project management skills.

Overall this project has allowed me to grow and improve as a researcher and an academic as well as providing a foundation for further research on this topic for myself and other researchers to increase the knowledge base around forensic entomology in aquatic environments. I look forward to working in the future on other projects using this research as a jumping off point.

2. Overview

2.1 Introduction to Decomposition and Forensic Entomology in Aquatic Environments

The science of forensic entomology relates to the use of insects in medicolegal investigations, usually for the purpose of estimating time since death (post-mortem interval, PMI_{min}) – the amount of time elapsed between death and the discovery of the body (Amendt, Krettek, & Zehner, 2004; Amendt et al., 2007). PMI_{min} is estimated by pathological methods (Goff 2010, George 2011) and mathematical models (Campobasso, Di Vella, & Introna, 2001) however invertebrates that have colonised human remains can be most precise (Amendt et al. 2004). Due to the close association between necrophagous insects and human (or animal) remains, it is possible to estimate PMI_{min} with a high degree of accuracy up to several weeks following the death of the individual (Amendt et al., 2004). Popular methods include estimating larval blowfly age (Campobasso et al. 2001) using thermal accumulation models, which uses flies' poikilothermic nature (Higley & Haskell, 2001) (i.e. their body temperature and development is primarily governed by environmental temperature (Ames & Turner, 2003; Warzecha et al., 1999)) and faunal succession in the later stages of decomposition (Amendt et al., 2004; Carvalho & Linhares, 2001), as this is linked to the stages of decomposition. Fauna include necrophagous, predatory, and parasitic insects as well as others which use the remains as part of their living environment (Amendt et al., 2004; Anderson, 2001b; Byrd & Castner, 2009; Campobasso et al., 2001; Greenberg, 1991; Voss, Spafford, & Dadour, 2009). Blow flies are often studied as they are the earliest colonisers of remains, thus providing the most useful indication of PMI_{min} (Greenberg 1991, Hewadikaram and Goff 1991, Dillon 1997, Anderson 2001, Amendt *et al.* 2004). The earliest recorded uses of what we might recognise as 'forensic entomology' can be dated back to c.1300AD in 'The Washing Away of Wrongs' by T'zu Sung, which details the investigation into the murder of a farmer in rural China (Benecke, 2001). However, much of the research which forensic entomologists rely upon today is much more modern with key studies beginning in the 1960s such as that by Payne (1965).

As discussed, it is possible to estimate PMI_{min} using a variety of different methods however a great deal of information can be gleaned by studying the insect life present on a cadaver (Amendt et al., 2004). As the earliest colonisers of remains, blow flies (Diptera: Calliphoridae) are the subject of many comprehensive studies and their lifecycles are generally well understood, although they differ from species to species (Erzinçlioğlu, 1996). In cases where the remains are easily accessible to blow flies colonisation is understood to occur very rapidly after death, often within minutes (Martín-Vega, Martín Nieto, Cifrián, Baz, & Díaz-Aranda, 2017). However, this fails to take into account the pre-colonisation phase (pre-CP); the time in between death and colonisation. George, Archer, & Toop (2013) note that the pre-CP cannot currently be estimated in Australia due to a lack of data, but in truth there is a lack of data relating to pre-CP across the board and a better understanding of this would improve accuracy of PMI_{min} estimations. While blow flies are typically the first to arrive on remains, the order of different insect species arriving throughout the decomposition period can also be used to estimate PMI_{min}, especially in the later stages of decomposition (Amendt et al., 2004; Anderson, 2001b; de Carvalho & Linhares, 2001; Greenberg, 1991; Hewadikaram & Goff, 1991; Higley & Haskell, 2009).

This colonisation by necrophagous insects is affected by numerous variables including temperature, humidity, presence of drugs in the decedent's system, and access to the remains (Amendt et al., 2004). Of these, temperature is known to be an important variable and as such the effect of temperature on different stages of the decomposition and insect colonisation process has been extensively studied. As it is an important component of estimating PMI_{min} from insect activity on remains, many studies have investigated development threshold temperatures (DTTs) as well as the influence of fluctuating temperatures on the development of common species of forensically important Diptera. This includes *Lucilia sericata* (Tarone, Picard, Spiegelman, & Foran, 2011; Wall, French, & Morgan, 1992), *Lucilia illustris* (Niederegger, Pastuschek, & Mall, 2010), *Calliphora vicina*, and *Calliphora vomitoria* (Ames & Turner, 2003; Defilippo & Bonilauri, 2013; Johl & Anderson, 1996), all of which are native to the UK, as well as species with importance outside the UK

including *Phormia regina* (Nabity, Higley, & Heng-Moss, 2006), *Sarcophaga haemorrhoidalis* (Byrd & Butler, 1998) and *Protophormia terraenovae* (Grassberger & Reiter, 2002). In the final part of this study (Chapter 7), the effect of temperature on oviposition behaviour was studied in *Calliphora vicina, Calliphora vomitoria* and *Lucilia sericata* in the laboratory. As this was a laboratory-based study access to the porcine liver (kept at ambient temperature) used as the oviposition substrate was not restricted, however in a field study or case research, this may not be the case. Under these conditions, *Calliphora vomitoria* and *Lucilia sericata* demonstrated the ability to oviposit at lower temperature thresholds of 16^{o}C and 17.5^{o}C respectively, and *Calliphora vicina* was able to oviposit at a lower threshold of 10^{o}C. However, field research conducted in Portsmouth, Hampshire, indicates that *Lucilia sericata* is able to oviposit at temperatures at least as low as 15^{o}C (pers. comm., unpublished data) which demonstrates that further research including field studies is important for fully understanding oviposition behaviour under different conditions. This includes building on the studies presented here to further investigate the effect of temperature in aquatic environments. In addition, during the laboratory study the adult flies were given 24 hours to acclimatise to the temperature being tested, and the temperatures were kept constant using a temperature-controlled insect growth chamber (IGC). In the wild, temperature fluctuations may have an effect on blow flies' ability to oviposit which may cause lower wild thresholds such as the 15^{o}C limit recorded during the aforementioned fieldwork conducted in Portsmouth. Other factors which may cause this discrepancy include geographical differences (the original study was conducted using colonies of laboratory-bred flies from parents wild-caught in Derbyshire, approximately 190 miles North of Portsmouth) and interaction effects of varying temperature, light levels, humidity, access to remains etc. While these factors have not been further explored as part of the studies presented in this thesis, access to remains in particular serves as a good example of one factor which impacts on insect colonisation of remains in water; the main focus of these studies.

While insect colonisation and decomposition have been extensively studied on land including bodies found above ground (Simmons, Cross, Adlam, & Moffatt, 2010;

Wang & Tsai, 1996; Wang et al., 2019), buried remains (Fiedler & Graw, 2003; Forbes, Stuart, & Dent, 2005; Mariani, García-Mancuso, Varela, & Inda, 2014; Pastula & Merritt, 2013; Schotsmans, Denton, et al., 2011; Schotsmans, Van de Voorde, De Winne, & Wilson, 2011; Szpila, Voss, & Pape, 2010; Tumer et al., 2013; Vass et al., 2008; Wilson et al., 2007) and bodies found in houses and vehicles (Al-Qahtni et al., 2019; Benecke, 1998; Goff, 1991; Voss, Forbes, & Dadour, 2008; Wang et al., 2019), there is a significant lack of research investigating decomposition and in particular insect colonisation of remains in aquatic environments. This is despite the global frequency of finding human remains in water whether through accidental drowning, as a result of natural means (for example damage to archaeological sites and cemeteries), recreational accidents, maritime accidents, body deposition in murder cases or suicide by drowning (Ahlm, Saveman, & Bjornstig, 2013; Ahmed, Rahman, & Van Ginneken, 1999; Caruso, 2011; Dietz & Baker, 1974; Evans, 2013 Haw & Hawton, 2016; Mateus, de Pablo, & Vaz, 2013; Peyron, Casper, Mathieu, Musizzano, & Baccino, 2018; Stoop, 2003). As might be expected, this is especially common in areas with easy access to water (Ahmed et al., 1999). One very recent case underscoring the need for this type of work is that of Lucas Dobson, a six-year-old whose body was found five days after he slipped into the River Stour in Kent while fishing with his family (BBC News, 2019). In this case the timing of the accident was known, however death may not have occurred for some time after and therefore is an unknown factor which requires a knowledge of aquatic taphonomic processes to investigate.

Other events such as aviation or boating accidents (Dumser & Türkay, 2008; Introna, Di Vella, & Campobasso, 2012), as well as mass disasters may result in human remains decomposing in the open sea, sometimes at extreme depths (Beauthier, De Valk, Lefevre, & De Winne, 2014; Dumser & Türkay, 2008; Ellingham, Perich, & Tidball-Binz, 2017; Heaton, Lagden, Moffatt, & Simmons, 2010) – although these bodies are not commonly recovered (Anderson & Bell, 2014). In addition to more open water environments (lakes, rivers, canals, oceans etc), human remains are sometimes also recovered from enclosed aquatic environments such as bathtubs (Devos, Timperman, & Piette, 1985; Pearn & Nixon, 1977; Peden, Franklin, Pearn, & Mahony, 2019; Schmidt & Madea, 1995b, 1995a; Wentworth, Croal, Jentz, Eshghabadi, &

Pluck, 1993; Yang, Choi, Lee, & Yoo, 2018), ponds and wells (Chin, Marwi, Jeffrey, & Omar, 2008; Dogan, Demirci, Erkol, Gulmen, & Deniz, 2010; Magni, Borrini, & Dadour, 2013; Sharma & Chandra Bajpai, 2013) or in one reported case, a toilet bowl (Wentworth et al., 1993) and another a septic tank (Lew, Bannach, & Rodriguez, 1996). Water bodies are also searched as part of missing persons cases by organisations such as The Water Search and Rescue Team (WSART) (Water Search and Rescue, 2019), and to locate other forensically important objects such as weapons (Parker, Ruffell, Hughes, & Pringle, 2010; Ruffell et al., 2017).

One reason for the lack of comprehensive literature on this subject are the myriad difficulties with conducting research in underwater environments. There are ethical and environmental considerations that researchers must be aware of; many floating or beached bodies in actual cases are discovered accidentally by beach-goers for example (Haglund & Sorg, 2002), necessitating careful experimental setups to avoid harm to members of the public. Depending on the country in which the research is to be undertaken there may be more or fewer restrictions imposed on where and how the research can be undertaken. In addition, the logistics of conducting this type of research are complicated and can quickly become time- and cost-prohibitive. Divers, vessels, and remotely operated vehicles (ROVs) equipped with cameras may be required for accessing experimental sites (Anderson & Bell, 2014; Ellingham et al., 2017; Schultz, Healy, Parker, & Lowers, 2013) but this can come with additional difficulties as divers may not always have good visibility (Parker et al., 2010) and other weather conditions may limit or prevent research taking place (Anderson & Bell, 2014). Divers may also not be able to access certain areas, for example if currents are too strong (Schultz et al., 2013). Body recovery in casework may involve draining the water body or trawling with grappling hooks, however these methods can be expensive, environmentally damaging, inefficient, or damaging to the evidence and/or crime scene (Parker et al., 2010). Other specialist equipment and personnel such as cadaver dogs, and geophysics & hydrogeophysics methods including sonar and ground penetrating radar (GPR) may also be used in casework (Parker et al., 2010), however these may be too costly, impractical, or otherwise unavailable for use in research.

Much of the research that has been conducted into maritime taphonomy originates from British Colombia, Canada (Ellingham et al., 2017). Researchers at Simon Fraser University have studied decomposition primarily in the marine environment at various depths using adult pig carcasses as a human analogue (Anderson & Bell, 2017; Anderson & Hobischak, 2004; Anderson & Bell, 2014; Anderson, 2008; Anderson & Bell, 2016). Notable differences were found in faunal scavenging and decomposition at the different depths. A carcass deployed at 300m was rapidly scavenged and skeletonised by very large numbers of amphipods while carcasses decomposing at 170m were primarily scavenged by larger crustaceans (Anderson & Bell, 2017). Another carcass decomposing at 300m was quickly consumed by several bluntnose sixgill sharks (Anderson & Bell, 2016). These carcasses in deeper waters did not pass through any of the typical stages of decomposition as they were consumed too quickly (Anderson & Bell, 2016), however at shallower depths 50% of carcasses floated for extended periods due to formation of adipocere following the bloat period (Anderson & Hobischak, 2004; Ellingham et al., 2017).

Despite the clear quality of these studies, the methodology is difficult to replicate as the studies were conducted using the Ocean Network Canada's Victoria Experimental Network Underseas (VENUS) observatory, a world-leading ocean observatory featuring cabled equipment including oceanographic sensors and cameras which operate continuously (Anderson & Bell, 2017; Ocean Networks Canada, 2019). A detailed description of the equipment is provided by Anderson & Bell (2014). This platform provides 'an un-paralleled method for studying undersea taphonomy in real-time' (Anderson & Bell, 2017) which clearly cannot be matched by research groups who do not have access to such high tech equipment. Using this equipment it was possible for the research group to light the area in which the carcasses were decomposing, and to record two-minute-long videos every 15 minutes without the need to use any battery-operated equipment (Anderson & Bell, 2016). Temperature, salinity, pressure, conductivity, density, and speed of sound were recorded using the oceanographic equipment (Anderson & Bell, 2017). In another study, the carcasses were monitored by divers (Anderson & Hobischak, 2004). Carcass recovery was

usually undertaken using an ROV with a specially designed lid to protect the carcasses (Anderson & Bell, 2016).

The methodology used for the field studies described in chapters 6 and 7 of this thesis was inspired by the methodology used by Anderson and colleagues in these studies from British Colombia, but lacking access to extensive and permanent marine biology or oceanography research facilities/equipment, modifications were made in order to make it possible to complete the study within practical constraints using equipment that may be more easily available. It is hoped that these methods could be used or improved on for future research including at other small laboratories worldwide.

2.2 Identifying Drowning, Submersion, and Decomposition in Aquatic Environments

Different descriptions of drowning exist, but it is usually considered to be a 'death within 24h of a submersion incident' (Byard, 2015), although so-called "dry drowning" (as of 2002 the use of this terminology is no longer recommended by the World Health Organisation (WHO)(McEwen & Gerdin, 2016)) can also occur. Various tests exist for diagnosing "true" drowning, which is usually considered to be 'inhalation of water which can pass the alveolo-capillary membrane and reach the circulation' (Piette & De Letter, 2006). One test which has been extensively discussed in the literature is the use of diatoms, unicellular algae which are present in almost all aquatic and damp environments (Horton, Boreham, & Hillier, 2006; McEwen & Gerdin, 2016; Vinayak, Goyal, Mishra, & Rai, 2010). In principle, if an individual is alive when they enter the water, diatoms can be aspirated during the drowning process and enter the circulatory system and other body tissue via the alveolar-capillary membrane (McEwen & Gerdin, 2016). It has been asserted that this test may be particularly useful in cases where heavily decomposed bodies cannot be diagnosed anatomically, and it may also be used to cross-reference water types and locations since many species are habitat-specific (Badu, Girela, Beltra, Ruz-caracuel, & Jimena, 2015; Horton et al., 2006). The test is controversial, particularly due to the potential for contamination and false-positives owing to the ubiquity of diatoms and other possible mechanisms aside from drowning for diatoms entering into organs (Badu et al., 2015; McEwen & Gerdin, 2016). Diatoms have variously been found in living

people and bodies not recovered from water, which suggests that they may enter the circulation during ingestion of food or inhalation (Krstic et al., 2002; McEwen & Gerdin, 2016). Passive contamination, in which diatoms enter the tissues of decomposing bodies due to hydrostatic pressure and relaxation of muscles, may also occur (Krstic et al., 2002). Conversely, diatoms may not be present in tissues from victims who are known to have drowned (Horton et al., 2006). This may be because of rapid death preventing diatoms from entering the organs (Krstic et al., 2002). Diatoms from clothing can also be analysed, however these can be acquired by individuals moving through water and can remain on clothing for some time afterwards (Peabody, 1999).

The use of diatoms to localise the site of drowning is described in two case studies from the United Kingdom (Horton et al., 2006). The first involved the body of a woman found in a river and the second involved the body of a boy found in a freshwater artificial pond. Control samples of diatoms from surface sediment in the local/regional area were compared to samples from the lungs and clothing of each cadaver. In the first case there was a statistically significant relationship between the lung & clothing samples and the control samples with specific sites along the river identified as being the closest matches. The second case was more complicated due to the lack of a water sample taken from the pond at the time of drowning, however it was still possible to identify many species from the lung samples that were also found in the pond water control samples. The results in each case were considered to support a diagnosis of death by drowning (Horton et al., 2006).

However, these methods are not necessarily useful in cases of other types of immersion death (e.g. “dry drowning”) (Piette & De Letter, 2006) or in cases where the decedent was dead before entering the water. Byard (2015) also claims that searching for diatoms to diagnose drowning has low success rates, although this is disputed by other authors for example Pollanen, Cheung, & Chiasson (1997), who found that diatom testing was useful in approximately one third of 738 freshwater drowning cases. Further to this, tests for diagnosing drowning do not help investigators to determine how long the decedent has been dead for (PMI_{min}) or how

long they have been in the water (including periods of full submersion and partial submersion as remains bloat and float to the surface of the water (Haefner, Wallace, & Merritt, 2004)), usually known as post-mortem submersion interval or PMSI. To add to the complication, these two figures are not always congruent with one another, for example in homicide cases where the victim was killed on land and later deposited in water, although there are instances in which PMI and PMSI may be equivalent if submersion occurs during the perimortem period. In addition, the stages of decomposition in water are known to be different to those on land (Dickson, Poulter, Maas, Probert, & Kieser, 2011), meaning that much of the existing taphonomic knowledge is irrelevant in aquatic scenarios. Authors have defined these stages as submerged fresh, early floating, floating decay, advanced floating decay, and sunken remains (Haefner et al., 2004; Hobischak & Anderson, 2002). Other signs of immersion such as "washer-woman's skin", foam around the mouth & nostrils, and the appearance of adipocere can be identified by a forensic pathologist, and there may also be other artefacts from the aquatic environment (such as mud, sand, aquatic weed or algae, and small aquatic fauna) present on or in the body (Papadodima, Athanaselis, Skliros, & Spiliopoulou, 2010). In shallow water, remains may go through the same stages of decomposition as remains on land, these being fresh, bloat, active decay, advanced decay, and skeletonization (Ellingham et al., 2017).

Alongside pathological and entomological information, these stages of decomposition are important when estimating PMSI, as are the large number of variables which alters these stages compared to the stages on land. As previously discussed, temperature and access to the remains are key factors, however due to the nature of aquatic environments other variables include salinity, water depth and the potential for the body to move in three dimensions, action of water currents and/or tides, aquatic bacterial communities and water chemistry, any water pollution, oxygen content and presence of aquatic scavengers (Dickson et al., 2011; Haglund & Sorg, 2002; Papadodima et al., 2010). Much of the information pertaining to these factors has been derived from case studies conducted in the USA (Wallace, Merritt, Kimbirauskas, Benbow, & McIntosh, 2008), Canada (Hobischak & Anderson,

2016; Petrik, Hobischak, & Anderson, 2013), and the Mediterranean (Pampín & López-Abajo Rodríguez, 2001; De Donno et al., 2014; Dumser & Türkay, 2008; González Medina, Soriano Hernando, & Jiménez Ríos, 2015; Magni, Borrini, et al., 2013; Magni, Pérez-Bañón, Borrini, & Dadour, 2013; Magni et al., 2014). Few case studies have been undertaken in the UK although Heaton et al. (2010) studied bodies recovered from a small number of UK rivers and canals. According to Henssge & Madea (2007), many of the studies concerned with estimating PMI lack practical relevance as they are not precise or reliable enough, or do not provide an immediate result. De Donno et al. (2014) extends this to include studies dealing with PMSI estimation, specifically those on human remains or animal models. This underscores the need for further research to be undertaken, including validation studies of existing methods (De Donno et al., 2014). Despite this claim, case studies are of clear importance for contributing to the body of knowledge, and several instances exist of case studies identifying species which have not previously been recorded on submerged remains or whose presence has previously not been used to provide PMSI estimations (Magni, Pérez-Bañón, et al., 2013; Magni et al., 2014; Wallace et al., 2008).

While there are aquatic arthropods which will feed on submerged remains (as well as terrestrial insects when they are able to gain access), there are usually considered to be no specialised necrophages among them (Haefner et al., 2004), although some possible exceptions to this have been recorded (Keiper & Casamatta, 2001; Magni, Pérez-Bañón, et al., 2013; Schuldt & Hershey, 1995; Wallace et al., 2008; Wartenberg, Reinhard, Nöllert, Staniczek, & Kupfer, 2017; Wipfli, Hudson, & Caouette, 1998). For example, Keiper, Chapman, & Foote (1997) describe colonisation of rat carrion in a woodland stream by midge larvae (Diptera: Chironomidae), and in this case patterns in colonisation were detectable. Similar results are reported by González Medina et al. (2015) and Myskowiak, Masselot, Fanton, & Schuliar (2010) who evaluated development rates of midges for PMSI estimation. As such, midges are an important indicator family for aquatic death scene investigation. In another study, Barrios & Wolff (2011) identified several indicators of different stages of decomposition, with benthic arthropods and neuston acting as indicators of submerged stages and

terrestrial insects (*Calliphora nigribasis* and *Oxelytrum discicolle)* indicative of floating stages. Due to this lack of specialised necrophages, different methods are often needed to estimate PMSI compared to using terrestrial insect life to estimate PMI on land.

Other research that may provide alternative methods for estimating PMSI is concerned with bacterial, mould, or algal succession. Mould and algae have been shown to play a role in decomposition of a variety of carcass types in aquatic environments (Chaloner, Wipfli, & Caouette, 2002; Haefner et al., 2004; Hobischak & Anderson, 2002; Johnston, MacIsaac, Tschaplinski, & Hall, 2004; Keiper & Casamatta, 2001; Keiper et al., 1997; Minshall, Hitchcock, & Barnes, 1991). Apart from using algae in the form of diatoms to diagnose drowning, algal species composition and frequency may be used to help determine PMSI (Haefner et al., 2004). This is sometimes referred to as forensic phycology (Keiper & Casamatta, 2001). Casamatta and Verb (2000, in Haefner et al., 2004) identified several species present on submerged rodent carcasses which had potential for use as an indicator of PMSI and in a study by Haefner et al. (2004) algal growth rates were strongly correlated with time since submersion. Since some aquatic invertebrate larvae are algivorous or herbivorous, it is worthwhile understanding the feeding behaviour of these larvae in order to understand or even predict which larvae may be present on remains (Keiper & Casamatta, 2001).

Bacteria have also been shown to play a crucial role in the decomposition process (Pechal et al., 2013). In recent years some studies have started to focus on the use of aquatic bacteria for PMSI estimation, for example Benbow, Pechal, Lang, Erb, & Wallace (2015) who were able to identify changes in epinecrotic bacterial communities on pig carcasses in a freshwater stream which could be used to estimate time since submersion. Similarly to using insect succession for PMI estimation, these bacterial communities vary according to region, season, land use and land cover (Benbow et al., 2015), and therefore similar studies need to be conducted in a variety of locations in order for the method to be most accurate. Pechal et al. (2013) also demonstrated seasonal and annual differences in communities of epinecrotic

bacteria on decomposing pig carcasses decomposing on land in a forested area. Bacterial activity was also found to be affected by access or exclusion of insect colonisers although this was highly variable (Pechal et al., 2013). Although this is not an aquatic study, it still demonstrates how a thorough knowledge of bacterial succession could be useful for estimating PMI across multiple environments. In a similar study but this time conducted across two freshwater streams, Lang et al. (2016) also demonstrated that epinecrotic bacterial succession could be used for estimating PMSI by associating it with time. Marine bacteria also colonised remains in a successional pattern in a study by Dickson et al. (2011). The authors also report several unique genera being found across three substrate sites on the pig carcass used, which may serve as an indicator of PMSI in a marine environment (Dickson et al., 2011).

Another study by Ueland, Breton, & Forbes (2013) demonstrated that adipocere formation did not occur on porcine tissue samples submerged in deionised water, but did occur on tissue samples in lake water. This was shown to be as a result of an abundance of gram-positive bacteria early in the post-mortem period, and was followed by a decrease in bacterial concentrations in the tissue over time (Ueland et al., 2013). This further shows the importance of bacteria in the aquatic decomposition process and in this case the formation of adipocere. Identifying the specific bacterial involved with this process would help to create a marker for adipocere formation in the aquatic decomposition process.

2.3 Oceanography, Movement of Bodies in Open Water, and Associated Post-mortem Damage

Another facet of underwater death scene investigation that has received increasing attention in recent years is that of oceanography and its use for predicting body displacement and likely recovery sites. Although this requires specific expertise not related to forensic entomology or taphonomy, it serves to demonstrate the interdisciplinary and inter-agency co-operation which is required for successful aquatic death scene investigation. Typically there has been a lack of co-operation between experts in these two fields arising from a lack of familiarity with the other

discipline (Ebbesmeyer & Haglund, 2002), however there is a growing awareness of the importance of both of these fields for body recovery from aquatic environments. When establishing the circumstances surrounding water-related death, it is important to establish where the body entered the water if this is unknown, or conversely where the body may exit the water if it is yet to be found (Mateus et al., 2013). Currents in open water can transport bodies hundreds of kilometres, such as in cases described by Blanco Pampín & López-Abajo Rodríguez (2001) in which bodies drifted as far as 420km from the point of entry into the water, Giertsen & Morild (1989) in which bodies drifted at least 500km, and Ellingham et al., (2017) in which bodies drifted up to 555km (300 nautical miles). Ocean current and water circulation maps for large bodies of water have been created for non-forensic purposes such as predicting fish migrations, however these can be also be used in maritime search and rescue (SAR) operations and forensic investigations (Mateus, Pinto, & Chambel-Leitão, 2015; Ruffell et al., 2017). One recent example of this is the use of computer simulations to predict the exit point of the missing Malaysia Airlines Flight MH370 (Ruffell et al., 2017). Such computer simulations have been shown to provide reliable predictions of body movement even in areas with complex currents (Ebbesmeyer & Haglund, 2002), although a model described by Mateus et al., (2015) failed to accurately predict the horizontal displacement of a body. Similar methods have also been used for predicting the movement of bodies in water and the time taken to travel a given distance (Ebbesmeyer & Haglund, 2002), for example in a study by Mateus et al. (2013) who used aerial imagery from Google Earth alongside known tidal conditions and sea surface temperatures to estimate the drift distances of two bodies off the coast of Portugal. These methods provide investigators with an area which can then be manually searched for remains (Ruffell et al. 2017), although certain environmental conditions may impede the reliability of predictions (Ebbesmeyer & Haglund, 2002).

When assessing the state of a body, it is important to distinguish between damage sustained during post-mortem body displacement and ante- or perimortem injuries which can be sustained for example when hitting the head on the bottom of a swimming pool (Papadodima et al., 2010). In the case of non-forensic remains (e.g.

archaeological skeletal matter) there may also be injuries present, and it is important to distinguish not only between damage sustained during body displacement and other injuries, but also between forensic and archaeological remains. However, this can be difficult especially in cases where adipocere formation slows decomposition and preserves remains (Ellingham et al., 2017).

Body displacement injuries are often to the head as cadavers in water usually lie face down at least initially, and the passive congestion of blood that this causes often leads to these injuries bleeding which can cause difficulties when diagnosing mode and time of death (Haglund & Sorg, 2002; Papadodima et al., 2010; Ruffell et al., 2017). Dangling limbs may also become abraded as the body moves along the bottom of the water system, with these injuries most commonly appearing on the toes and palms of the hands, and abrasive or corrosive modification of exposed skeletal matter can also occur (Haglund & Sorg, 2002). In addition, post-mortem injuries can be caused by aquatic faunal feeding behaviour, such as in a case reported by Colombage & Telisinghe (2010) in which a body was recovered from the South China Sea with damage to the ears, lips, heart and lungs caused by feeding fish and crustaceans. Other examples include skin lesions caused by the amphipod *Niphargus elegans* (Vanin & Zancaner, 2011), and damage to remains by crayfish (although in this case the location of the damage on the body and the absence of current in the recovery area prevented the injuries from being confused with displacement-related damage) (Duband, Forest, Clemenson, Debout, & Péoc'h, 2011).

In several cases, crayfish feeding damage has also been confused with other types of trauma, for example in a case described by Duband et al. (2011) in which police initially identified lesions on the body of a 60-year-old woman as being as the result of a possible criminal assault. In another case, crayfish feeding damage on the body of a young girl was confused for human bite marks and the boyfriend of the decedent's mother spent more than 15 years on death row before being exonerated (Wallace, 2019). Another case from Padua, Italy, is reported by Pascali, Viel, Cecchetto, Piagaiani, Vanin, Montisci & Fais (2019). Here, several crayfish were found

on and around the body, and at autopsy three further crayfish were found inside the thoracic cavity.

Damage by feeding cookie cutter sharks (Isistius spp.) is also well-documented, for example in a case reported by Ribéreau-Gayon, Rando, Schuliar, Chapenoire, Crema, Claes, Seret, Maleret and Morgan (2016) in which victims of the Yemenia airplane crash displayed characteristic circular lesions which are consistent with the bites of cookiecutter sharks, some of which had tooth marks at the edges. These lesions have also been reported in a drowning victim found in a bay in Japan, and C-shaped lesions were also noticed which were presumed to be incomplete bite marks (Hayashi, Higo, Orito, Ago & Ogata, 2015). The authors also report that the lesions were also found on another body and on the body of a pygmy sperm whale (*Kogia breviceps*), both found in the same bay. Interestingly the shark had not previously been observed in this bay, however the authors purport that the shark may now inhabit the bay due to global warming (Hayashi et al. 2015). The same circular and C-shaped lesions were found in another Japanese case reported by Makino, Yachihara, Ageda, Arao, Fuke and Miyazaki (2004), and in a case in Hawaii reported by Ribéreau-Gayon, Carter and Regan (2018). In this case it was possible that the shark activity occurred peri-mortem, and in fact cookie cutter shark predation of live humans has even been recorded in another case between Hawaii and Maui where a swimmer suffered two bites resulting in the characteristic round wounds (Honebrink, Buch, Galpin & Burgess, 2011). Other shark bits are also recorded on human remains and it is possible in some instances to determine shark size from the bite characteristics (Lowry, Fagundes de Castro, Mara, Whitenack, Delius, Burgess & Motta, 2009).

2.4 Whale Fall Studies, Shipwreck Studies, and their Potential for Contributing to Aquatic Forensic Ecology Knowledge

As previously discussed, studying decomposition and invertebrate colonisation on remains in aquatic environments is fraught with difficulties. This research is ongoing with authors contributing to the body of literature worldwide, but alongside this there are other avenues which have not been extensively explored.

Haglund and Sorg (2002) suggest shipwreck literature as one possible avenue for increasing knowledge of the behaviour of human remains in water, and discuss the recovery of 15 bodies from the sunk Belgian cargo ship, the *Mineral Dampier,* as described by Kahana, Almog, Levy, Shmeltzer, Spier and Hiss (1999, cited in Haglund and Sorg, 2002). In this study, the authors noted decompositional changes to the remains as they were recovered, which took between two and 433 days. The post-mortem changes noted include washerwoman's hands, bloating, marbling and skin slippage in the earlier post-mortem period, through to total saponification and skeletonization in the four bodies recovered 433 days post-mortem. Here the authors concluded that, due to differences in their observed rates of adipocere formation compared to other case studies, adipocere formation alone should not be used for estimating PMI.

Two more historic shipwrecks are discussed by Ebbesmeyer and Haglund (2002). The first is concerned with the 1875 collision of the paddle wheeler *Pacific* with the clipper *Orpheus* off the coast of Washington State, USA. While the *Orpheus* survived largely intact, the *Pacific* sank killing almost all of the passengers and crew. Here, bodies and wreckage from the collision travelled up to 100 miles (~160km) and were recovered during the course of 34 days. Interestingly, storms in the area resulted in the majority of the remains and wreckage being carried inland instead of further out to sea (Ebbesmeyer & Haglund, 2002). One of the bodies was recovered scalded, leading investigators to believe that an explosion may have occurred during the sinking of the *Pacific*. In fact, explosions and fire damage are not unknown in boating accidents due to using petrol as fuel, and arson may also occur as a method of disposing of an unwanted boat (Becker, 2013).

The second case discussed by Ebbesmeyer & Haglund (2002) is that of a ferry, the *Clallam*, which ran into difficulty with a gale after departing Port Townsend, Washington in January, 1904. As the ship began taking on water, the Captain ordered all women and children into lifeboats, however due to the bad weather the lifeboats were overcome by waves and all the passengers drowned. After some hours of trying to keep the *Clallam* afloat help arrived in the form of a tug, however after 12 miles

the tug also ran into difficulty and cut the towline. Several men remaining on board were then swept into the water by the waves, and finally the *Clallam* sank. At least 55 of the 89 passengers and crew members were killed, of which 32 bodies were recovered over the course of approximately 14 days (there is one body for which the precise recovery date is unknown) (Ebbesmeyer & Haglund, 2002).

Across both of these incidences, the majority of the bodies were recovered in a relatively small area, providing key information for any future searches for remains, wreckage or other forensic evidence that might occur on this particular stretch of coastline. Of the approximately 250-300 lives that were lost in these wrecks, only about 6% of the bodies were recovered, further demonstrating the difficulty of body recovery in this type of environment (Ebbesmeyer & Haglund, 2002). Using knowledge of currents in the area as well as information reported in contemporary newspaper articles, it was possible for the speed at which wreckage from the *Pacific* drifted inland to be estimated, and this estimate was found to be comparable with estimates made from modern oceanographic measurements (Ebbesmeyer & Haglund, 2002). This was also comparable to the drift speed of an oil spill which occurred in 1989 (Ebbesmeyer & Haglund, 2002), and as such serves as a useful predictor for drift speeds in subsequent cases of shipwrecks, oil spills, or other similar events which might occur in the area.

One highly relevant shipwreck study is that described by Introna et al. (2012). The *Kater Radez I* motorboat sank during a naval blockade in March 1997 in the Mediterranean Sea. On board were approximately 120 Albanian illegal immigrants attempting to land in Italy. 34 people survived however 58 migrants, mostly women and children, were confirmed dead, and many more were missing. Due to the depth at which the sunken boat came to rest (800m), it was not possible to recover bodies using divers and as such two of the bodies were recovered by ROV, however Interpol DVI protocol was followed for the recovery of the boat and any bodies remaining inside. The bodies recovered by ROV were fully skeletonised and exhibited evidence of feeding activity by marine fauna, likely crustaceans, shrimps and small fish. The bodies found within the boat were in varying states of decay, and some marine

scavengers including gastropods and crabs were found inside the boat. Despite the long PMI, the bodies were not completely skeletonised. This is in contrast to the *Mineral Dampier* case study discussed above, in which three of the bodies were fully skeletonised (Introna et al., 2012). In this case it appeared that the closed compartments within the boat coupled with multiple layers of heavy clothing helped to protect the bodies from scavenging by larger fauna, except for in areas such as the head and hands which were not covered by clothing. The two bodies recovered by ROV were fully skeletonised, lending additional weight to this idea.

Olivieri et al. (2018) describe the sinking of another migrant boat off the coat of Italy near Lampedusa which resulted in a large number of fatalities. In this case a fishing boat with around 500 Eritrean, Ethiopian and Somali migrants sank after a fire broke out and the boat capsized. Of these 500 migrants, 155 survived, 194 bodies were recovered shortly after the capsize, and a further 108 bodies were later recovered from inside the hull of the boat (Bertoglio et al., 2019). Little information is available about the state of the bodies on recovery, however it was possible to identify a number of the victims through both anthropological and odontological methods, and DNA analysis (Olivieri, Mazzarelli, Cappella, De Angelis, Piscitelli & Cattaneo, 2017).

In light of recent incidences of migrants attempting to gain entry to Europe via the Mediterranean, knowledge obtained from other shipwreck studies is highly relevant and of global interest. Ellingham, Perich, & Tidball-Binz (2017) report that maritime fatalities in the Mediterranean more than doubled between 2015 and 2017 and the majority of bodies are never recovered. The authors purport that this is in part to do with a lack of understanding of marine taphonomy, further underscoring the need for research to be undertaken both in the Mediterranean and elsewhere. Since the majority of bodies are not recovered, it is difficult to know exactly how many people may have died in these events, but the current UNHCR estimate for number the of dead and missing migrants as of August 2019 is 839 while the estimate for 2018 is 2277 (UNHCR: The UN Refugee Agency, n.d.). The agency Missing Migrants reports that 1.5% of attempted crossings resulted in death in 2018 and while many of the victims are of an unknown region of origin, the next most numerous are from Sub-

Saharan Africa (Missing Migrants Project, 2019). Fewer NGO boats are now available for rescue operations, which the UN cites as being the cause of increased numbers of deaths of migrants attempting the crossing (Crisp, 2018). This is an ongoing issue which underscores the need for a thorough understanding, not only of decomposition and anthropophagy in aquatic environments, but also of movement of bodies in marine environments and likely exit points of remains.

In addition to shipwreck studies, there is a huge amount of potential in whale fall studies for understanding both post-mortem changes to remains in water and which organisms may be found on remains recovered from extreme depths. Historically many whale fall studies were undertaken on whale carcasses discovered by accident, although more recently it has been possible to place suitable carcasses at drop sites for study purposes, mostly in the deep ocean (Smith & Baco, 2003). Similarly to aquatic forensic entomology, there are relatively few whale fall studies (Sumida et al., 2016) and these large carcasses decompose differently to human carcasses or smaller mammals (Anderson & Bell, 2016), however they still provide additional data which can be used to bolster understanding of decomposition and faunal succession in aquatic environments.

According to Schäfer (1972, cited in Haglund and Sorg, 2002) the bones of deceased whales disarticulate prior to the skin rupturing, and then fall individually from the body once rupturing occurs. A similar sequence of events has been noted in a study of decomposing human remains in the Salish Sea (Haglund, 1993 cited in Haglund and Sorg, 2002). In this case parts of the body falling from the major body unit became scattered, complicating the recovery process. In deep-ocean environments, these fallen whale carcasses form species-rich habitats which consist of many specialist organisms such as bone-eating worms, limpets, sipunculids (peanut worms), hagfish, and sleeper sharks (Glover et al., 2013; Smith & Baco, 2003). Due to the large size of whale carcasses, they can have a lasting effect on faunal communities, and can inform other marine ecological studies (Smith, Bernardino, Baco, Hannides, & Altamira, 2014). While these so-called ‘whale-fall species’ may not all be relevant to decomposition of human remains in marine environments, it is

possible that crossover exists, although a greater volume of research is necessary to fully understand the potential similarities. However, lysianassid amphipods have been recorded feeding on whale carcasses in large numbers (Smith et al., 2014), and this same family along with other families of amphipod have also been observed feeding on other carcass types in marine forensic ecology studies including human (Dumser & Türkay, 2008) and pig (Anderson & Bell, 2017).

In addition to whales, other carcass fall studies such as sharks (Higgs, Gates, & Jones, 2014), porpoises (Kemp et al., 2006) and invertebrates such as jellyfish (Sweetman, Smith, Dale, & Jones, 2014; Yamamoto et al., 2008) may yield additional information and are worth being aware of.

2.5 Research Questions

Four main research questions are investigated in the studies contained within this thesis:

1. Is there an operational need for research into decomposition and insect succession in aquatic environments?
2. Which insects and invertebrates (terrestrial and aquatic) colonise remains decomposing in fresh and salt water in the South of England?
3. Can a recognisable pattern of insect/invertebrate succession be identified in any of the aquatic environments tested?
4. Can any indicator species be identified which would help investigators estimate PMI_{min} in any of the aquatic environments tested?

Question 1 is addressed primarily in chapter 3, although other research relating to operational need is discussed throughout.

Question 2 is addressed in chapters 4, 5, and 6, where records of insect and invertebrate species observed or collected from different aquatic systems are provided. The environments considered are:

1. Enclosed freshwater and saltwater environments
2. A natural saltwater harbour environment

3. A manmade freshwater pond

Questions 3 and 4 are also addressed in these same chapters, with further discussion provided in chapter 8.

Other themes which are discussed throughout the thesis include methodological difficulties of investigating decomposition and insect succession in aquatic environments, factors affecting insect colonisation in aquatic environments and how these relate to factors affecting decomposition in terrestrial environments, and the differences in taphonomy between different aquatic environments.

2.6 Theoretical and Methodological Components of the Research

The research presented here is primary research in the form of a questionnaire and interviews with CSIs, forensic entomologists, and other professionals relevant to death scene investigation, alongside field-based experimental work. A previously published study conducted in the laboratory is used to illustrate the way in which extreme temperatures may affect the ability of blow flies to oviposit on remains.

The research draws on existing knowledge of insect succession, and decomposition in aquatic environments. Although some research similar to this project has previously been conducted, this study provides data for the Portsmouth area, and is unique in England. Through questionnaires, interviews, and literature searching, the operational need for such research to be undertaken is discussed.

A detailed discussion of the methods for each study can be found in their respective chapters, however this section seeks to present an overview of each of the methods used alongside a discussion of how the different studies link together.

2.6.1 Forensic Entomology and Underwater Death Investigation: A Review of its Utilisation and Potential

This study was designed to investigate whether there is an operational need for the field studies presented here, and others like them, to take place (see question 1 in

section 2.5 Research Questions above). To assess the current use of forensic entomology in aquatic death scene investigation, a literature review was first undertaken using the EBSCO Discovery Service search engine. In total, over 3000 articles appeared as a result of the initial key term search. The most relevant articles were then selected based on relevance, date of publication, and viability for informing the forensic community.

In addition to the literature review, first responders and forensic experts completed a questionnaire tailored to their specialism and aimed at ascertaining their opinions on various aspects of underwater death investigation. Finally, to consolidate the data and provide a better awareness of context, senior police investigators took part in semi-structured interviews. This allowed the review of their experiences of underwater death investigation from a management perspective, as all three of the participants have currently or previously been responsible for deployment of resources for scenes.

The results of this study show that further research to fill gaps in understanding of aquatic forensic entomology and underwater death investigations is necessary. As such, several field studies were designed to collect some preliminary data for the South of England.

2.6.2 Influence of Two Enclosed Water Types on Entomological Species Colonisation in Portsmouth, UK

The first of these field studies investigated decomposition of rabbit carcasses in translucent plastic boxes in an urban garden environment. One carcass was placed into a box containing freshwater and a layer of sediment from a local stream, and the other was placed into a box containing seawater and a layer of sand from the coast nearby. Each was then allowed to decompose naturally, and water temperatures and insect activity were monitored. The purpose of this study was method development and to provide some baseline data for the types of species that might be found in a larger scale study (discussed in section 2.6.4 below).

The research questions addressed in this study are questions 2, and 3: which insects and invertebrates colonise remains decomposing in water in the South of England, and can a recognisable pattern of insect/invertebrate succession be identified in this environment? It was possible to build a picture of which terrestrial insects are likely to be present on remains in these enclosed aquatic environments, although the results should not be generalised beyond their intended scope of providing baseline data on the insect species which were likely to be found in the follow-up larger scale study. As it concerns insect succession, the timing of appearances of different insect species is consistent with previously published data. In this study no aquatic species were recorded and therefore only succession among terrestrial species is addressed.

2.6.3 A Preliminary Investigation of Faunal Colonisation of Remains in Open Water

Following on from the previous study, two larger scale field studies were designed in order for the results to have better ecological validity. The first study presented here consists of method development for investigating invertebrate colonisation on remains in open water, specifically a harbour environment. The methodology was informed by other existing studies as outlined above but was designed to be appropriate for studying decomposition in water on a small scale, without the use of specialist equipment such as divers or ROV-mounted cameras. In this way, aquatic research is made more feasible for smaller laboratories, student studies, and laboratories without easy access to open water. The research was conducted on the University of Portsmouth's Marine Research Raft using stillborn piglet (*Sus scrofa domesticus*) carcasses.

In the first method, a barrel-shaped crayfish pot was modified by removing the one-way entrances at either side (see chapter 5, section 5.3). A piglet carcass was placed inside the modified pot which was then secured shut using zip ties to form a cage. Initially, three such cages were prepared and placed into the water at different depths – two were attached to a metal frame suspended from the raft resulting in the uppermost carcass resting just below the water line, and the lower of the carcasses being submerged by approximately 76cm. The third cage was suspended from the raft by way of a chain, attached to an anchor and lowered to the sea floor,

at a depth of between 4 and 9m depending on tidal action. The cages were removed from the water and checked for faunal activity approximately once per week for three months, at which point all three carcasses were fully skeletonised. This design was then repeated a second time, with only the two cages attached to the metal frame. Finally a follow-up trial was undertaken in which only the cage anchored at the sea floor was used, but this time with the addition of a GoPro Hero 4™ camera and Blink Time Lapse Controller™ in order to capture still frame photographs every 30 minutes as the carcass decomposed.

The second method tested used a standard 20 litre plastic whelk pot to house the piglet carcass. The carcass was secured to the inbuilt bait hook by way of zip ties. The pot was weighted with concrete (inbuilt), attached to the raft via a length of rope and chain, and lowered to the sea floor. The pot was removed from the water and checked for faunal colonisation approximately once every two weeks between 10th November 2017 and 1st February 2017.

The third and final method involved a creel shaped lobster pot to house the carcass, also fitted with the GoPro™ camera and Blink Time Lapse Controller™. Again a chain was attached to the pot to secure it to the raft, and the pot was lowered to the sea floor. Monitoring of faunal scavenging was primarily undertaken using the GoPro™ images, however the pot was also periodically removed from the water between 26th January 2018 and 12th February 2018 for checking.

This section considers several of the main research questions for this project. Firstly question 2 (which insects and invertebrates colonise remains decomposing in fresh and salt water in the South of England?) was addressed. While this study is primarily focussed on method development it was nonetheless possible to collect some preliminary data on faunal colonisation of the remains in this harbour environment. This was most successful when using the GoPro™ as this allowed for organisms to be observed feeding which were no longer present when the remains were removed from the water. Secondly, the feeding organisms were monitored for any recognisable succession pattern (question 3) however none was observed. Some

previously published research indicates this is likely to be the case in aquatic environments, however due to the paucity of data on faunal succession in aquatic environments, this still warrants investigation. Lastly, it was also not possible to identify any indicator species which would help with PMI_{min} estimation in this environment (question 4) as the majority of the species observed were ubiquitous, and those that appeared more sporadically were incidental to the remains. In addition, this study is lacking in repeats and therefore does not have a large amount of data to draw on.

2.6.4 A Checklist of Arthropods Associated with Piglet Carcasses Decomposing in a Freshwater Pond Environment in Southeast England

Alongside the previous study, faunal succession on remains in a freshwater pond environment was also investigated. A small pond was dug in an area of private woodland near Wickham, UK. The pond was filled with tap water and left to mature. A cover made from heavy wooden planks and chicken wire was designed to prevent access to the pond by scavengers. A piglet carcass was placed into the pond and allowed to decompose naturally while colonising insect species were monitored. Once the piglet was fully decomposed it was removed from the pond, and to ensure that all remains were collected the pond was drained and the debris was sifted for any remaining skeletal matter. The pond was then allowed to refill with rainwater. For comparison purposes, a dog crate was set up on dry land nearby to house a second piglet carcass which was allowed to decompose concurrently. Again, insect colonisation was recorded. This was repeated four times.

This study considers research questions 2, 3, and 4 in a similar manner to the harbour-based study. It was possible in this case to compile a list of the colonising insect fauna in both the pond and land environments (question 2) and in the land environment appearance of early colonisers was consistent with previously published research. However, due to this research being only in the preliminary stages as concerns decomposition in aquatic environments in the UK, and due to the relatively small number of replicates, this study overall does not attempt to comment on successional patterns (question 3). Some species were observed which could

potentially act as indicator species in the pond environment (question 4), however more research is required to say with confidence which species, if any, could be useful in this way.

2.6.5 Effects of Environmental Temperature on Oviposition Behavior in Three Blow Fly Species of Forensic Importance

The final study presented in this thesis investigates temperature-related oviposition behaviour in three species of blow flies in the laboratory. Although this study was not designed to investigate oviposition behaviour in water, it still serves to demonstrate the importance of temperature on oviposition behaviour which is known to be a factor in both terrestrial and aquatic environments. In addition, it is useful to have an awareness of oviposition behaviour under extreme or unusual conditions including both extreme temperatures and aquatic conditions.

Here, 20 adult male and 20 adult female flies of the same species were placed into each of three Bugdorms™ and provided with sugar, water, and porcine liver. The Bugdorms™ were then placed into an insect growth chamber at 20^{o}C for 24 hours after which the presence/absence of eggs was recorded. This protocol was then repeated at subsequent 5^{o}C intervals either side of 20^{o}C until the flies would no longer oviposit. Where oviposition was occurring at one temperature and not the next, finer increments of 2.5^{o}C and 1^{o}C were used. In addition to recording the presence or absence of eggs after 24 hours, the quantity of eggs (or sometimes larvae) at higher temperatures was estimated by placing a 1cm^{2} grid over the top of the liver and counting the number of squares which contained at least one egg or larva when observed directly from above. At the end of the 24 hr oviposition period, the liver with the eggs or larvae was transferred onto a thin layer of sawdust in a clear plastic tank, and eggs were reared through to the pupal stage. The resulting number of pupae in each tank was recorded and a series of statistical tests were used to investigate survival.

3. *Forensic Entomology and Underwater Death Investigation: A Review of its Utilisation and Potential*

3.1 Foreword

Before moving on from the study into temperature-dependent blow fly oviposition behaviour to any aquatic field studies, it was important to determine whether there was a genuine operational need for this research to be carried out. As such, a questionnaire was designed to evaluate the opinions of different experts and personnel who may be present at an aquatic death scene on the current use and utility of forensic entomology for this type of investigation. Following this, a focus group was carried out with SIOs to obtain a more thorough visualisation of aquatic forensic entomology in an operational and investigational context.

3.2 Statement

Article title: Forensic Entomology and Underwater Death Investigation: A review of its utilisation and potential

Authorship details: Ody, H., Smith, P., Brown, K.

Publication outlet: International Journal of Police Sector Management or Journal of Forensic Investigation

Current status: In preparation

The questionnaire for this paper was designed and carried out by the student. The semi-structured interviews were carried out by Dr Paul Smith. The paper was written as a collaborative effort by the student and Dr Paul Smith.

3.3 Abstract

This paper presents a review of the literature and a survey of practitioners and investigation leaders to establish the current scope and utilisation of forensic entomology in immersion death investigations. The study, based on the South Coast of England, engages with local law enforcement personnel and international expertise in forensic entomology to (1) establish the current scope of entomology on land and in water, and; (2) identify gaps in practice to optimise research and research

dissemination to the benefit of forensic practice. The intention is to bridge the gap between the forensic scientist, the Crime Scene Investigator and Senior Investigating Officers. The outcomes suggest that there is considerable evidence available to aid the investigation, but it is not utilised to its full potential. Practitioners welcomed further research but emphasised the need for better engagement between the forensic scientist and investigator to optimise use in immersion deaths and support practitioner awareness. Though the findings are based on the south coast of England, the outcomes and details of the review have considerable potential to forensic practitioners worldwide.

Keywords:

Decomposition; Death Investigation; Forensic entomology; Immersion death investigations;

3.4 Introduction

At some point in their career, it is possible that forensic practitioners will manage scenes and evidence from immersion or underwater death investigations. Routinely, they will utilise the forensic and investigative sciences to identify the victim, reconstruct the circumstances surrounding the death, and in some cases, establish links to other individuals involved. However, where the remains are submerged, perhaps displaced by tides and currents, or purposely obscured, there is an added complexity and a requirement to use the appropriate scene and evidence morphology, along with a range of environmental factors, to reconstruct the transit of the body and piece together the circumstances as accurately as possible. This will involve the intrinsic factors affecting the decomposition of the body (for example, body size, whether the body is covered, etc.) along with the extrinsic elements, such as temperature, water type, water flow, and faunal or entomological activities in and around the body itself.

In regards to the use of forensic entomology in immersion deaths and in underwater decomposition in general, the point of departure for this paper is that there are some common misconceptions or, sometimes, a lack of awareness regarding the full gamut

of evidence available from underwater environments. The principal reason for this is the apparent dearth of research and reports from casework in areas relating to underwater death investigations and the use of underwater entomology. It is sometimes assumed that most trace evidence is washed away, adversely affected by the submerged environment, and, it is, perhaps, of limited value. Alternatively, often practitioners have a fundamental awareness of the basic facts but are not aware of the full scope of the evidence available or of the need to read the environmental context in each situation to support wider interpretations of the data available. The current literature demonstrates that vital evidence can and does persist even when there is an advanced stage of decomposition or disarticulation of the body. However, there are questions regarding how much of this actually reaches practitioners, where new and more applied research is required, and how it should be disseminated.

Therefore, this paper seeks to review the use of forensic entomology in relation to immersion death investigations, and the associated decomposition of the remains in underwater environments. The principal objective is to inform practice through an up-to-date detailed literature search and review, providing a critical analysis of existing thinking and practice considerations. This includes an overview of forensic entomology and a review of the intrinsic and extrinsic decompositional factors in the underwater environment. This is compared to a previous literature review conducted by Merritt and Wallace (2001), which found that more than 85% of studies into faunal colonisation of human remains were concerned with terrestrial fauna, while only 15% considered the possible role of aquatic organisms.

The paper then provides a practitioner viewpoint on underwater death investigation through a questionnaire (with frontline practitioners) and semi-structured interviews with Senior Investigating Officers. The triangulated results provide an overview of current practice in the South of England, much of which has relevance for investigators in other jurisdictions. The literature review and the experience of relevant practitioners helps to form a level of optimisation of the existing data, guiding future studies to provide a usable reference with a focus on practice.

3.5 Method

A review of relevant literature was conducted on 14th August 2019 and began with a search for publications using the EBSCO Discovery Service search engine. Terms including “immersion death forensic”, “underwater death investigation”, “forensic entomology underwater”, “underwater forensic search” were used generating over 3000 articles and papers. Choosing the correct database for this kind of task is imperative for ensuring that the correct results are located. In this case EBSCO Discovery Service was chosen as a suitable database due to its comprehensive nature and ease of use. After an initial review of the titles, abstracts and themes, selection of the most salient articles based on relevance, date of publication and viability in regards to informing the forensic community provided 184 publications for the review. The outcomes of which produced four defined themes including:

- scope of underwater death investigation;
- the application of forensic entomology;
- intrinsic decompositional changes in the underwater environment;
- extrinsic factors affecting decomposition, including oceanography, movement of bodies in open water, and associated post-mortem damage;

In addition to the review of the literature, first responders and forensic experts completed a questionnaire tailored to their specialism. A questionnaire was selected to collect this data due to the low cost, and the ability to easily reach a large number of participants worldwide. The design of the questionnaire ascertained the opinions of experts involved in (a) the initial response to immersion death scenes, for example, experienced crime scene examiners, police investigators, and (b) practicing forensic entomologists. A letter of introduction outlining the aims of the study was provided alongside a consent form and contact details for the research team. We utilised a purposive sample of the population concentrating on crime scene practitioners, and those practicing forensic entomology throughout the world. The questionnaires were distributed via email and participants were asked to complete questionnaires bespoke to their role. Participants selected their own role from a list (see appendix 5) and the questionnaire was aimed at professionals who were either forensic

entomologists or another forensic or related profession, but not both. Where participants were accessed through an organisation, a gatekeeper was used to separate the researcher from any potential respondents. Participants who had not attended aquatic death scenes were also included in the study, as it was important to understand different perspectives and experiences including from practitioners with different levels of involvement with aquatic death scene investigation. In total, 32 participants completed the questionnaires (20 female, 11 male and 1 not specified), 8 forensic entomologists and 24 crime scene / investigation personnel. Binomial testing indicated no evidence that the proportion of female to male respondents was different to expected (P=.085). Of the 8 Forensic Entomologists, 3 were male and 4 were female (Figure 1), and their experience in the role was between 10 and 18 years of practice. Of the CSI respondents: 16 were female and 8 were male (Figure 2); all were crime scene investigators, or senior crime scene investigators; experience in the role ranged from 2.5 years to over 23 years, and; 18 worked in the South of England, 2 from the Midlands region of England, 1 in the North of England and 1 from Scotland (Figure 3).

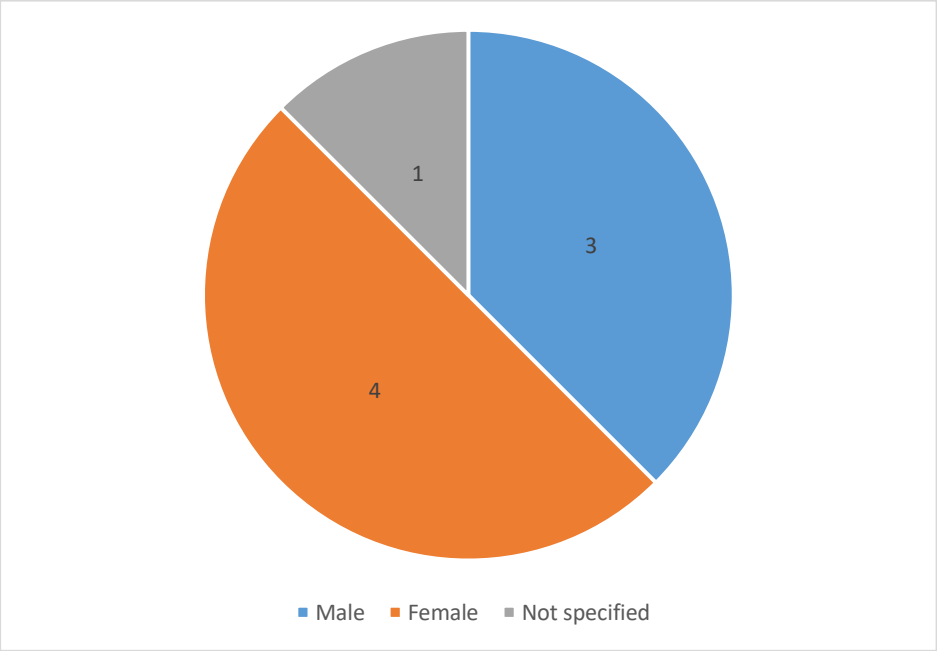

Figure 1: Gender breakdown of forensic entomologists

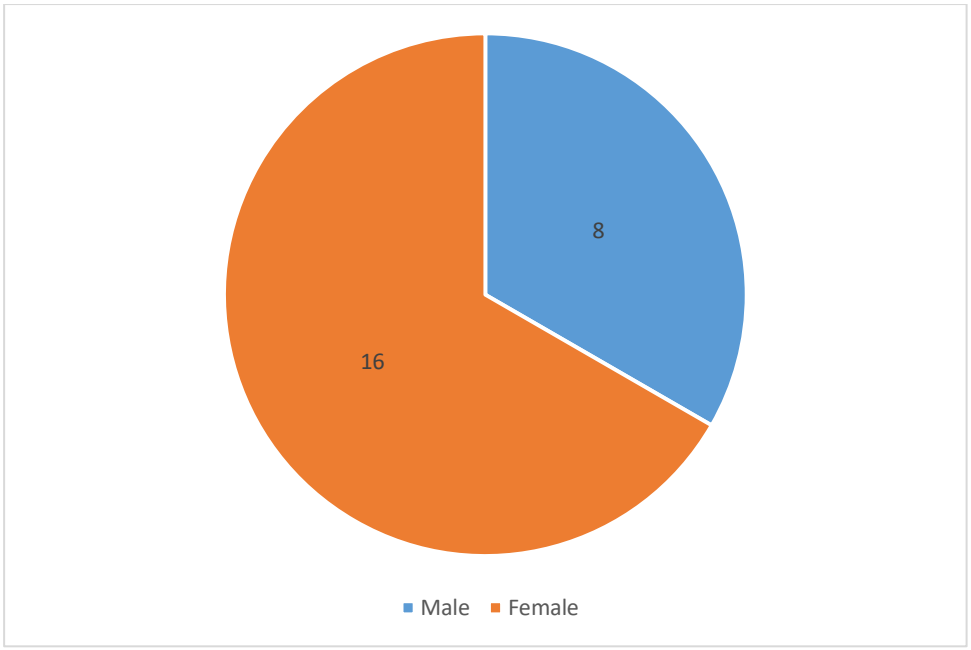

Figure 2: Gender breakdown of CSIs

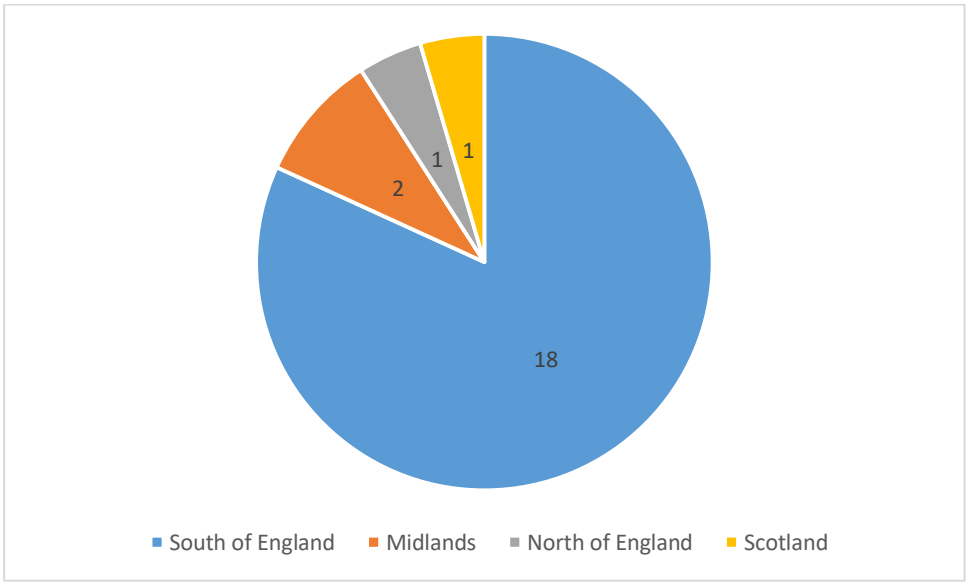

Figure 3: CSIs' Region of Work

The questionnaire explored the number of death scenes and immersion death scenes attended, or alternatively, for the forensic entomologists, the evidence they had received and analysed from immersion death scenes. Following this, the questionnaire explored participants' awareness of forensic entomology, or, for the forensic entomologists, the types of entomological evidence processed from death scenes and immersion death scenes. Finally, the questionnaire provided the

opportunity for participants to reflect on the use of entomology and its general application.

To consolidate the data and provide a better awareness of the contextual issues affecting immersion death investigations, semi-structured interviews arranged with senior police investigators asked them to reflect on their experience in underwater death investigations. In this case participants were asked to provide consent to be interviewed and to have their comments included verbatim in this article. The aim of the semi-structured interview was to review the experiences of the participants during the recovery of bodies, or after the investigation of water related death scenes. The semi-structured format allowed the researcher to ask more open-ended questions as well as asking questions based on responses given by the interviewees. The interviews gathered the views and experiences of 3 senior investigators in one UK coastal police force, all of which had over 15 years' experience in a senior investigative role. The interviews gave the participants the opportunity to discuss their considerations regarding underwater death investigation, including any perceived gaps, good practice and views on where research could assist in the future. We undertook descriptive analysis of the questionnaires, and qualitative data analysis of all interview responses using thematic analysis, triangulating the review of literature, the questionnaire and interview outcomes. The objective was to show the predominant trends and thoughts of participants, relating this, where applicable, to the literature review and generating a model to guide practice based on the accumulated salient points of reference.

3.6 Results and Discussion

3.6.1 Review of Literature

Comparison to other databases

In this case, EBSCO Discovery Service was chosen as a suitable database to conduct the literature review. However, many other databases exist which could be considered suitable for this task. One of the important factors when deciding which database to use is the number of results returned using particular search terms. A

search using database which returns fewer results is more likely to be missing key articles while a database which returns a vast number of results is likely to have more irrelevant content which must be filtered out before analysis. Using the initial search terms, EBSCO Discovery Service returned over 3000 results. Using the same search terms, Science Direct and JSTOR return similar numbers of results (over 3000 and around 2500 respectively) however PubMed returns under 250 results and Web of Science returns under 200 results. These results must then be assessed according to the chosen parameters (in this case results were assessed based on relevance, date of publication and viability for informing the forensic community, as discussed in the Method section above). In the case of EBSCO Discovery Service this resulted in 184 articles which were used in the final literature review. The other databases mentioned in this section did not undergo this process, however it is likely that Science Direct and JSTOR would result in a similar number of usable results while PubMed and Web of Science would clearly yield far fewer results.

Comparison to a previous literature review

A previous literature review by Merritt and Wallace (2001) found that an overwhelming majority of relevant literature considered the role of terrestrial rather than aquatic organisms in forensic investigation. In contrast, our literature review focused on aquatic death scene investigation as a whole and not exclusively the role of aquatic organisms in forensic investigations. It also did not consider any literature pertaining to the role of terrestrial fauna in forensic investigations. However, it is clear from the results that research into aquatic death scene investigation (and by implication aquatic faunal succession) is lacking and still requires more attention. Merritt and Wallace (2001) conclude that forensic scientists and police should have a thorough understanding of the aquatic organisms that may be found on human remains and the environmental factors that have an impact on the distributions of these organisms. These conclusions are also borne out by our literature search, as discussed below.

Scope

The UK has approximately 11,073 miles of coastline, 22 major rivers and countless smaller rivers, streams, lakes, ponds, canals, and reservoirs. In these waters, between the year 2015 and 2018, there were 2469 registered water related fatalities, 849 of these attributed to suicide, 36 related to crime and the rest were accidental or by way of natural causes (National Water Safety Forum, 2015, 2016, 2017, 2018). Of the water fatalities registered, around 46% occurred at sea, including the coast and harbours, and 54% occurred in inland waters. To put this in context, looking specifically at the year 2017, there were 607,172 deaths in the UK, of which 141,313 were "avoidable" (Office for National Statistics, 2017). In the same year, there were approximately 579 'avoidable' water fatalities, which accounts for less than 0.5% of the avoidable deaths in the UK, in that particular year (National Water Safety Forum, 2017). Globally, the frequency of finding human remains in water has been documented by Ahlm, Saveman, & Bjornstig (2013), Ahmed, Rahman, & Van Ginneken (1999); Caruso (2011); Dietz & Baker (1974); Evans (2013), Haw & Hawton (2016), Mateus, de Pablo, & Vaz, (2013), Peyron, Casper, Mathieu, Musizzano, & Baccino, (2018), and, Stoop (2003). Events such as aviation or boating accidents (Dumser & Türkay, 2008; Introna et al., 2012), as well as mass disasters may result in human remains decomposing in the open sea, sometimes at extreme depths (Beauthier et al., 2014; Dumser & Türkay, 2008; Ellingham et al., 2017; Heaton et al., 2010). In addition to more open water environments (lakes, rivers, canals, oceans etc.), human remains are sometimes recovered from enclosed aquatic environments such as bathtubs (Peden et al., 2019), wells (Dogan et al., 2010; Magni, Borrini, et al., 2013) or in one reported case, a septic tank (Lew et al., 1996).

Recent global trends are relevant too, particularly where it influences the number of water related fatalities. In recent years, many refugees and migrants have lost their lives crossing the Mediterranean (Last & Spijkerboer, 2014). According to the United Nations High Commissioner for Refugees (UNCHR), between the year 2015 and 2018, there have been over 1.7m people arriving in the European Union (EU) crossing the Mediterranean Sea, with over 14,000 missing or dead (UNHCR: The UN Refugee Agency, n.d.). Kovras and Robins explored the management of migrant bodies by

national and EU authorities. They provide some reinforcement regarding the need for an appropriate investigative approach, using all available evidence to identify the bodies (Ellingham et al., 2017; Kovras & Robins, 2016). They observe that DNA testing is routinely included, but there are "limited efforts" to identify individual bodies (Kovras & Robins, 2016). A policy report by the United Nations in 2018 acknowledged this, stating, "Those that are found will likely never be identified" (Villagran, 2018). They also acknowledge a myriad of logistical difficulties constraining identification in this context.

The logistical and practical difficulties notwithstanding, many fundamental methods of identification and reconstruction are limited here, due to "ad hoc practices" undertaken by the different authorities involved (Villagran, 2018). This does indicate a lack of consistency and compatibility in methods, capacity and capability, which is much more of a global issue (Ribaux, Walsh, & Margot, 2006). The management and identification of the dead involves stages of retrieval, transportation, post-mortem examination, storage and repatriation. The use of forensic science methods and evidence to support the identification process is relevant to all five stages (Ellingham et al., 2017; Mediterranean Missing, 2016). This could include reconstruction and using evidence to provide intelligence on the provenance of the remains. For example, looking at tidal patterns and currents (Ellingham et al., 2017; Mateus et al., 2013); using entomological or underwater faunal activity and environmental conditions to provide time of deposition, transit and minimum post-mortem interval (Ambade et al., 2013; Amendt et al., 2004; Ellingham et al., 2017; Payne, 1965); and, using physical anthropology and odontology methods to aid personal identification (Byers, 2016; Ellingham et al., 2017) in addition to DNA and fingerprints, when available. However, the intricacies of the relationship affecting decomposition underwater is complex and very different to that in land-based scenes. It is apparent, that though there is significant opportunity to use forensic evidence to provide both reactive and proactive (intelligence) information, the resources available determine the level of investigation and the broader investigative strategy for immersion death (this is discussed in more detail in the discussion).

Decomposition and its Effect

As outlined above, not all water related deaths are the result of drowning. Knight and Saukko (Knight & Saukko, 2004) highlight that bodies recovered from water could have died naturally before or after falling into the water. They may have died from injury before or while being in the water, or they may have died from immersion without drowning. Diagnosis of drowning, or the general cause of death, can be far from straightforward and a reliable history may not always be available (Byard, 2015). Understanding the process of decomposition and the impact of the environmental factors on the body are essential to not only help determine the location of key evidence, but also help to piece together the relevant ante-mortem, peri-mortem and post-mortem activities.

Stages of decomposition differ between land and water. Payne (1965) described six stages of decomposition on land consisting of fresh, bloated, active decay, advanced decay, dry, and remains. Other authors have described variations on these stages, sometimes not distinguishing between active decay and advanced decay, leaving out the bloat stage, or leaving out the remains stage (Anderson & VanLaerhoven, 1996b; Reed, 1958; Rodriguez, 1982). In comparison, Payne and King (1972) first described the stages of decomposition in water as submerged fresh; early floating; floating decay; bloated deterioration; floating remains; and sunken remains. More recently, Hobischak and Anderson (2002) and Haefner et al. (2004) redefined the stages of decomposition in water as submerged fresh; early floating; floating decay; advanced floating decay; and, sunken remains. This is apparent in case studies for example Heaton, Lagden, Moffatt, & Simmons (2010).

These stages of decomposition in water can help investigators provide an estimate of minimum post-mortem interval (PMI_{min}), or as is more likely in an aquatic environment, post-mortem submersion interval (PMSI) – in other words the length of time that the body has been in the water. In many cases PMSI, PMI and floating time of a corpse in water can be difficult to determine (Magni et al., 2014), and some crossover may exist between the terms. However, as with PMI on land, it is possible to estimate PMSI based on insect evidence, although the factors, which affect insect

colonization of remains in water and on land, are different. One such factor is the observable sink/float sequence as bodies may float or sink upon entry to the water, remain submerged for up to three weeks in cold water (MacDonell & Anderson, 1997), and then float again as gases build up during the bloated or early floating stages (Dickson et al., 2011). While the body is floating, there is the potential for colonisation by terrestrial insects including blowflies, often used as early indicators of PMI_{min} on land (Papadodima et al., 2010). Therefore, it is necessary to understand the interplay of these many different factors including the stages of decomposition in water, the characteristics of the water itself, and the behaviour and lifecycles of both terrestrial and aquatic invertebrates in order to estimate PMSI accurately.

To add to the complication, the two figures for PMI_{min} and PMSI are not always congruent with one another, for example in homicide cases where the victim was killed on land and later deposited in water, although there are instances in which PMI and PMSI may be equivalent if submersion occurs at the time of death, during the peri-mortem period. In addition, as already highlighted, the stages of decomposition in water are known to be different to those on land (Dickson et al., 2011), meaning that much of the existing decomposition or taphonomical knowledge is irrelevant in aquatic scenarios. These stages can be difficult to identify and occur more as an ongoing process than discrete phases. Other signs of immersion such as “washer-woman’s skin”, foam around the mouth & nostrils, and the appearance of adipocere can be identified by a forensic pathologist, and there may be other artefacts from the aquatic environment (such as mud, sand, aquatic weed or algae, and small aquatic fauna) present on or in the body (Papadodima et al., 2010). In shallow water, remains may go through the same stages of decomposition as remains on land, these being fresh, bloat, active decay, advanced decay, and skeletonization (Ellingham et al., 2017).

Extrinsic Factors Affecting Decomposition in Underwater Environments

In regards to the extrinsic factors, temperature and access to the remains are key. Other variables to consider, include salinity; water depth; the potential for the body to move in three dimensions; the action of water currents and/or tides; aquatic

bacterial communities and water chemistry; whether there is any water pollution; oxygen content and presence of aquatic scavengers (Dickson et al., 2011; Haglund & Sorg, 2002; Papadodima et al., 2010). Much of the information pertaining to these factors has been derived from case studies conducted in the USA (Wallace et al., 2008), Canada (Hobischak & Anderson, 2016; Petrik et al., 2013), and the Mediterranean (De Donno et al., 2014; Dumser & Türkay, 2008; González Medina et al., 2015; Magni, Borrini, et al., 2013; Magni, Pérez-Bañón, et al., 2013; Magni et al., 2014; Pampín & López-Abajo Rodríguez, 2001). Few case studies have been undertaken in the UK although Heaton *et al.* (2010) studied from a small number of UK rivers and canals. According to Henssge & Madea (2007), many of the studies concerned with estimating PMI lack practical relevance as they are not precise or reliable enough, or do not provide an immediate result. De Donno *et al.* (2014) extends this to include studies dealing with PMSI estimation, specifically those on human remains or animal models. This underscores the need for further research to be undertaken, including validation studies of existing methods (De Donno et al., 2014). Despite this claim, case studies are of clear importance for contributing to the body of knowledge, and several instances exist of case studies identifying species which have not previously been recorded on submerged remains or whose presence has previously not been used to provide PMSI estimations (Magni, Pérez-Bañón, et al., 2013; Magni et al., 2014; Wallace et al., 2008).

There are organisms that will feed on submerged remains (as well as terrestrial insects when they can gain access) but there are no specialised necrophages and as such, it is different to using terrestrial insect/invertebrate life to estimate PMI on land. Because of this, it is important to investigate different potential methods – such as bacterial succession or use of moulds and algae (Chaloner et al., 2002; Haefner et al., 2004; Hobischak & Anderson, 2002; Keiper & Casamatta, 2001; Keiper et al., 1997; Minshall et al., 1991).

Another facet of underwater death scene investigation that has received increasing attention in recent years is that of oceanography and its use for predicting body displacement and likely recovery sites. Although this requires specific expertise not

related to forensic entomology or taphonomy, it serves to demonstrate the interdisciplinary and inter-agency co-operation that is required for successful aquatic death scene investigation. Typically, there has been a lack of co-operation between experts in these two fields arising from a lack of familiarity with the other discipline (Ebbesmeyer & Haglund, 2002), however there is a growing awareness of the importance of both of these fields for body recovery from aquatic environments. When establishing the circumstances surrounding water-related death, it is important to establish where the body entered the water if this is unknown, or conversely where the body may exit the water (Mateus et al., 2013). Currents in open water can transport bodies hundreds of kilometres, such as in cases described by Pampín & López-Abajo Rodríguez (2001) in which bodies drifted as far as 420km from the point of entry into the water Giertsen & Morild (1989) in which bodies drifted at least 500km, and Ellingham *et al*., (2017) in which bodies drifted up to 555km (300 nautical miles). Ocean current and water circulation maps for large bodies of water have been created for non-forensic purposes such as predicting fish migrations, however these can be also be used in maritime search and rescue (SAR) operations and forensic investigations (Mateus et al., 2015; Ruffell et al., 2017). One recent example of this is the use of computer simulations to predict the exit point of the missing Malaysia Airlines Flight MH370 (Ruffell et al., 2017). Such computer simulations provide reliable predictions of body movement even in areas with complex currents (Ebbesmeyer & Haglund, 2002), although a model described by Mateus *et al*., (2015) failed to predict the horizontal displacement of a body accurately. Similar methods are used for predicting the movement of bodies in water and the time taken to travel a given distance (Ebbesmeyer & Haglund, 2002), for example in a study by Mateus *et al*. (2013) who used aerial imagery from Google Earth alongside known tidal conditions and sea surface temperatures to estimate the drift distances of two bodies off the coast of Portugal. These methods provide investigators with an area to manually search for remains (Ruffell et al., 2017), although certain environmental conditions may impede the reliability of predictions (Ebbesmeyer & Haglund, 2002).

When assessing the state of a body, it is important to distinguish between damage sustained during post-mortem body displacement and ante- or peri-mortem injuries which can be sustained for example when hitting the head on the bottom of a swimming pool (Papadodima et al., 2010). In the case of non-forensic remains (e.g. archaeological skeletal matter) there may also be injuries present, and it is important to distinguish not only between damage sustained during body displacement and other injuries, but also between forensic and archaeological remains. However, this can be difficult especially in cases where adipocere formation slows decomposition and preserves remains (Ellingham et al., 2017).

Body displacement injuries are often to the head as cadavers in water usually lie face down at least initially, and the passive congestion of blood that this causes often leads to these injuries bleeding which can cause difficulties when diagnosing mode and time of death (Haglund & Sorg, 2002; Papadodima et al., 2010; Ruffell et al., 2017). Dangling limbs may also become abraded as the body moves along the bottom of the water system, with these injuries most commonly appearing on the toes and palms of the hands, and abrasive or corrosive modification of exposed skeletal matter can also occur (Haglund & Sorg, 2002). In addition, post-mortem injuries can be caused by aquatic faunal feeding behaviour, such as in a case reported by Colombage & Telisinghe (2010) in which a body was recovered from the South China Sea with damage to the ears, lips, heart and lungs caused by feeding fish and crustaceans. Other examples include damage to remains by crayfish (although in this case the location of the damage on the body and the absence of current in the recovery area prevented the injuries from being confused with displacement-related damage) (Duband et al., 2011), and skin lesions caused by the amphipod *Niphargus elegans* (Vanin & Zancaner, 2011).

The intention of the review of literature was to explore the existing thinking regarding entomology and related taphonomy correlated with immersion death investigations. The review shows there are a vast amount of variables to consider, covering several areas of specialism. Each water context demands alternative considerations and presents an array of independent factors that will affect accurate

interpretations of events linked to the death. However, there are significant gaps regarding entomological activities in the underwater environment, additionally the research available is isolated to certain geographical areas and very little in the UK for example. The aim of the review was to inform, identify the 'highlights' and provide context to practitioners, also the objective was to inform the questionnaire and interviews with the senior investigating officers and provide a framework to identify future research requirement.

3.6.2 Findings From the Questionnaires

Crime Scene Investigator Response

Of those responding to the survey (n=24), 16 were female and 8 were male. Binomial testing indicated no evidence that the proportion of female to male respondents was different to expected (p=.085). The age range for participants was 26-52 and the mean age was 41 (SD=7.83). The participating CSIs had attended over 2400 death scene between them. This includes sudden deaths of varying causes and therefore not all required a major investigative function. In regards to attendance at immersion deaths, 5 out of the 25 respondents had attended a scene involving a body in water during their career, with 1 respondent attending around 80 immersion death scenes. In total, the number of immersion deaths attended was 104 between all the CSIs. As figure 4 outlines, around 4% of the deaths attended have been immersion deaths, with 3% of all deaths providing entomological evidence, which was recovered, but in 10% of death scenes, insect evidence was observed but not recovered. Therefore, where the recovery of entomological evidence took place, there was inevitably an investigative requirement to do so.

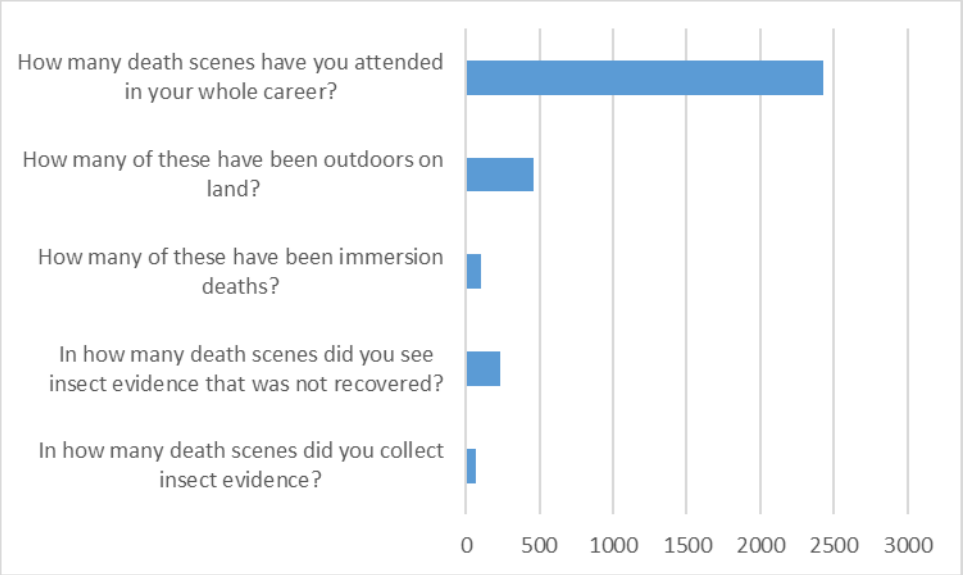

Figure 4: Shows the number of CSI death scenes attended in their career, which includes the number of outside scenes, immersion death scenes and scenes where the observation of, and / or recovery of insect evidence took place.

The CSI perception of forensic entomology and their reflection on knowledge and awareness of its potential use to investigations varied, as outlined in figure 5. 46% (11) acknowledged a moderate understanding of its utility, whereas 25% (6) felt they had a thorough and comprehensive understanding of entomology and 17% (4) reported that they had a good understanding of the use of insect evidence in investigations. Of the 6 recording a "thorough and comprehensive understanding", they attended between 35 and 200 death scenes in their careers, however, there were 12% (3) rating themselves as knowing little about forensic entomology. All 3 of these participants had little involvement with death investigations generally. This questionnaire did not include questions about participants' level of education or involvement with training programmes, as this was not the focus of the study, however previous studies have highlighted the inequalities in training and education in other areas of forensic investigation (National Academy of Sciences, 2009; Magni, Guercini, Leighton, & Dadour, 2013) which may explain the variance in levels of understanding. In regards to whether or not forensic entomology is adequately used, which is outlined in figure 7, the majority 46% (11), felt it was used an acceptable amount, or that it was used to its full potential, with 27% (8) feeling it was somewhat underused, and one person feeling it was badly underused. Of the respondents, 34%

(8) had been personally involved in collecting insect evidence, and 43% (10) had engaged with a forensic entomologist at some point in their career.

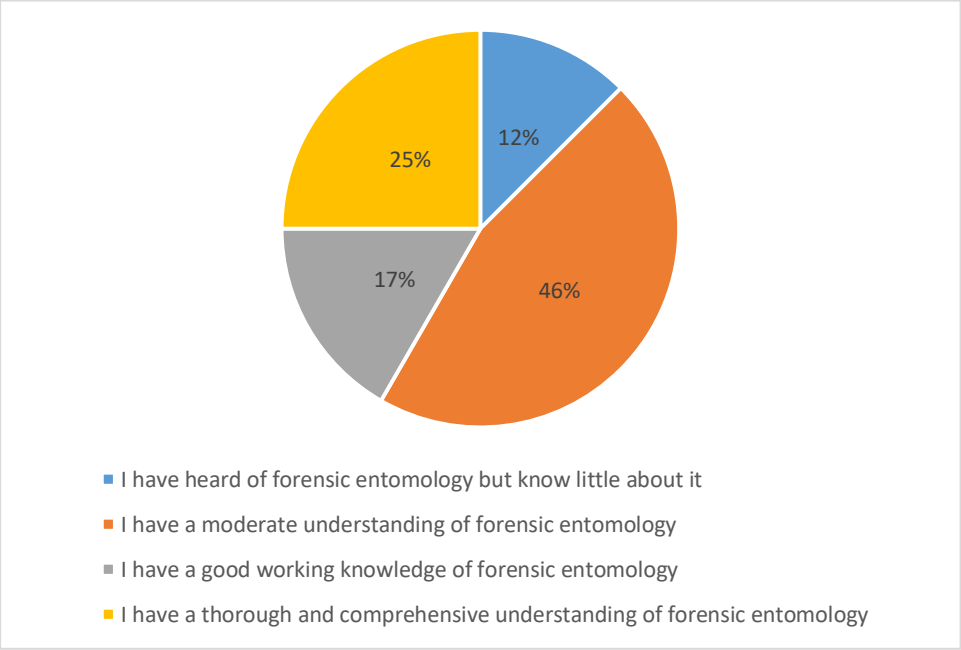

Figure 5: Outlines CSI awareness of forensic entomology

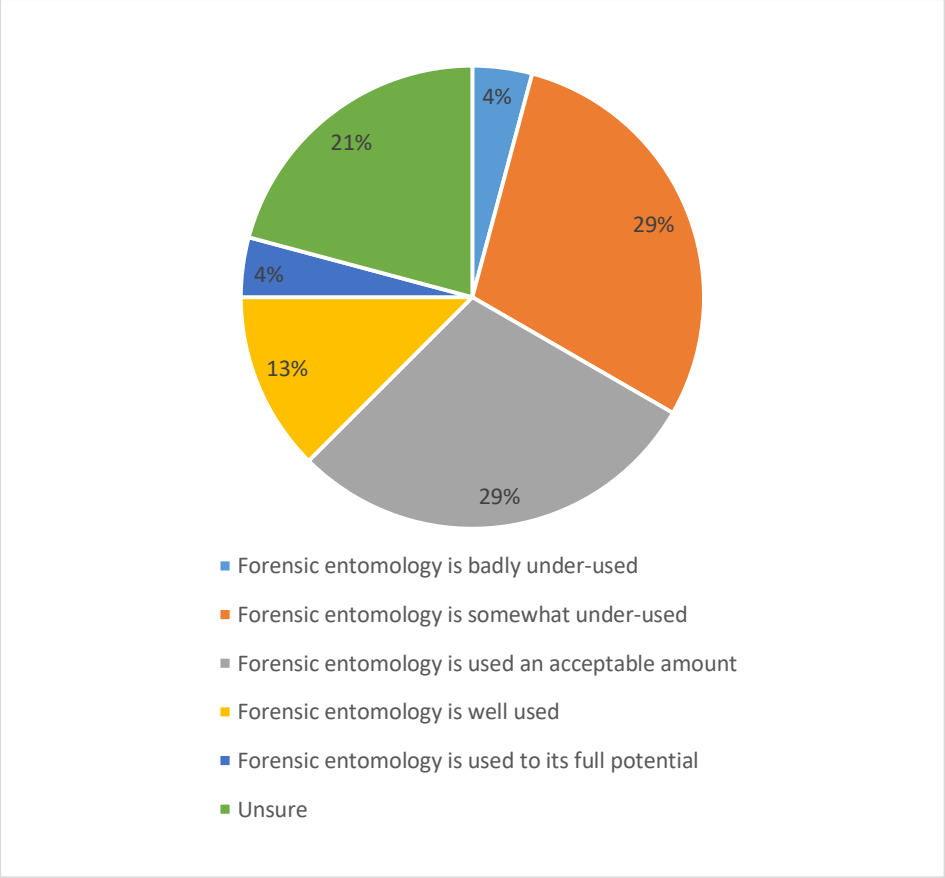

Figure 6: Outlines CSI opinion on whether forensic entomology is used to its potential

Forensic Entomologist Response

Eight forensic entomologists responded to the questionnaire, which included many of the working experts in UK and Europe at the current time. Between them they had been involved in over 500 cases, 184 (37%) of which were outdoors and on land. Understandably, as with the CSIs, the experience of processing entomological evidence from immersion deaths or water related scenes was far less, with only 3% (17) of cases coming from water related death scenes. This is presented in figure 7. Due to the small number of responses, inferential statistical analysis was considered unsuitable. The forensic entomologists documented the types of insects encountered in outdoor scenes and water based scenes, outlined in figures 8 and 9 respectively. As expected, the recovery of Dipteran related samples was more prominent in outdoor death scenes, but less in immersion death cases. However, as

highlighted in the literature review, this is common in partially submerged remains through either floating or being only partially submerged. Aquatic species, including Ostracoda, crayfish and Mollusca have been recovered from immersion death scenes, also of note is one forensic entomologist noting Ostracoda appearing in an outdoor sample.

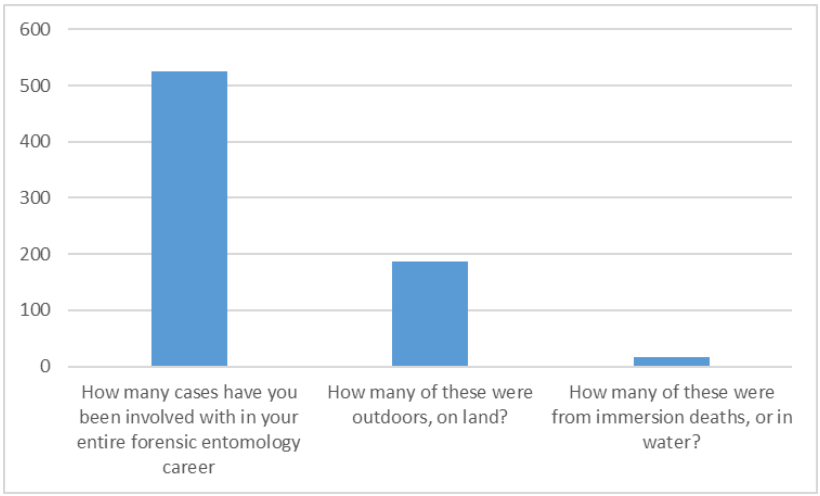

Figure 7: Outlines how many cases the forensic entomologists have been involved with in their career, including outdoor scenes and immersion deaths.

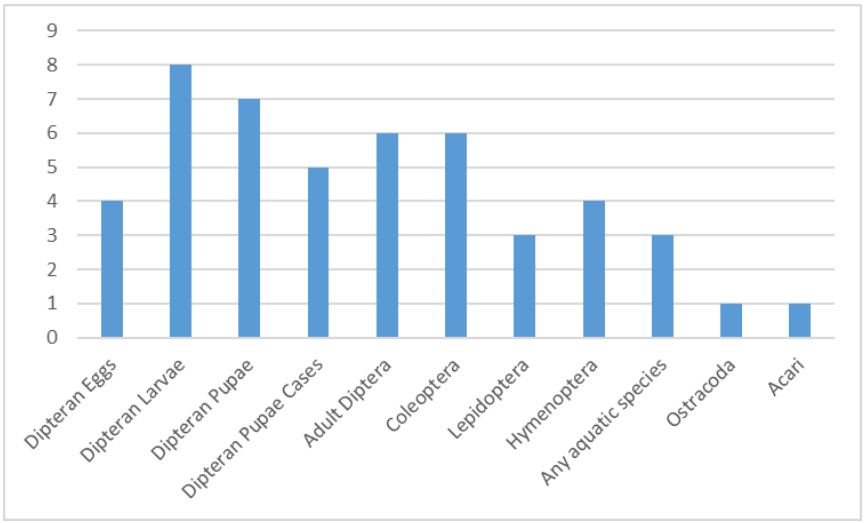

Figure 8: Outlines responses to the types of insect encountered by the forensic entomologist from outdoor death scenes

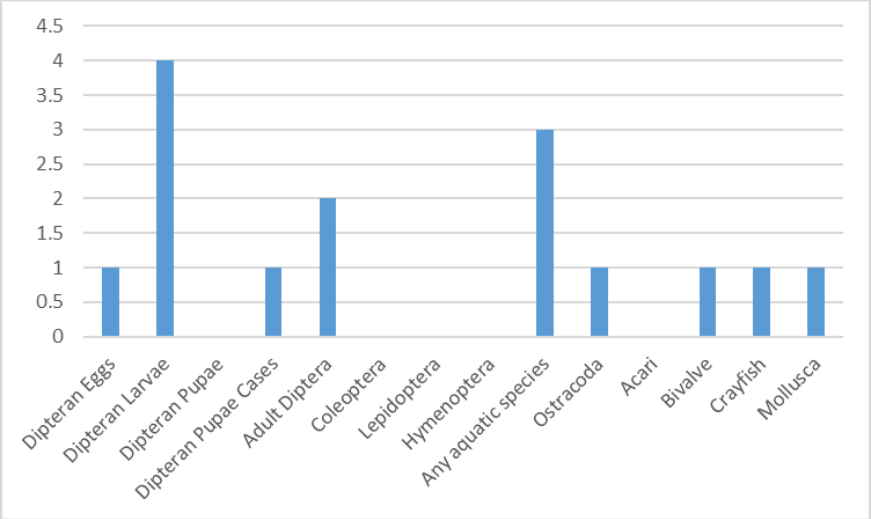

Figure 9: outlines responses to the types of insect encountered by the forensic entomologist from immersion or water related death scenes

The forensic entomologists were asked to rate the quality of samples they received from third parties, this is presented in figure 10. From the responses, half of the participants received the evidence from a third party, including the forensic pathologist, CSI, Police Investigator, etc. The other half recovered the samples themselves. On many occasions, the forensic entomologist does not attend the scene, and therefore relies on the evidence from the third party and relevant case documentation. In the majority of cases, the quality of the samples was adequate, with one response stating that it was poor with the integrity of the evidence and any extrapolation compromised. In figure 11, the forensic entomologists outlined whether or not they felt forensic entomology is used to its full potential. Half of the participants felt it was underused, and the other half felt it suitably used. The natural deduction from this is that the use of forensic entomology varies between the individual experiences of the entomologist; the next section looks at this in more depth.

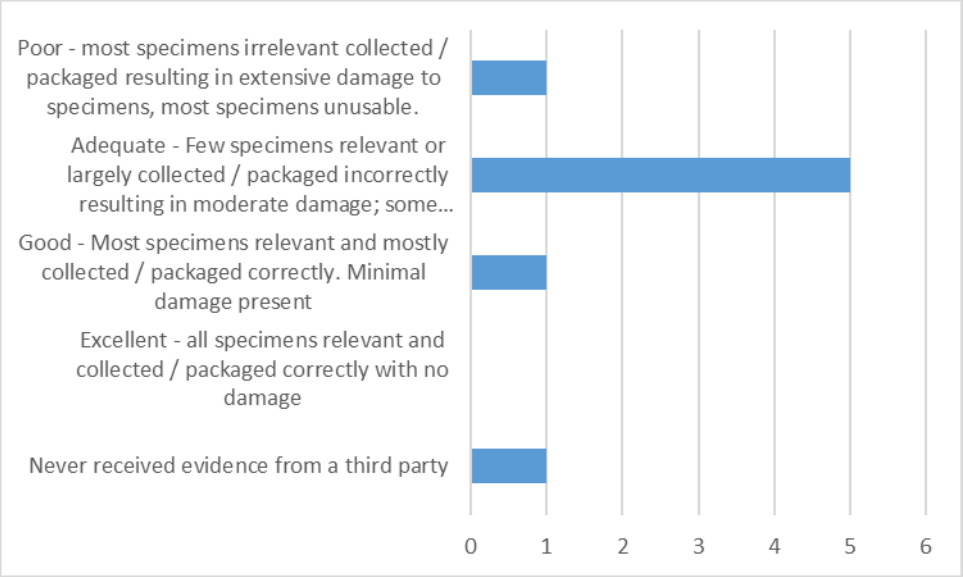

Figure 10: outlines the forensic entomologist view on the quality of the samples received from third parties.

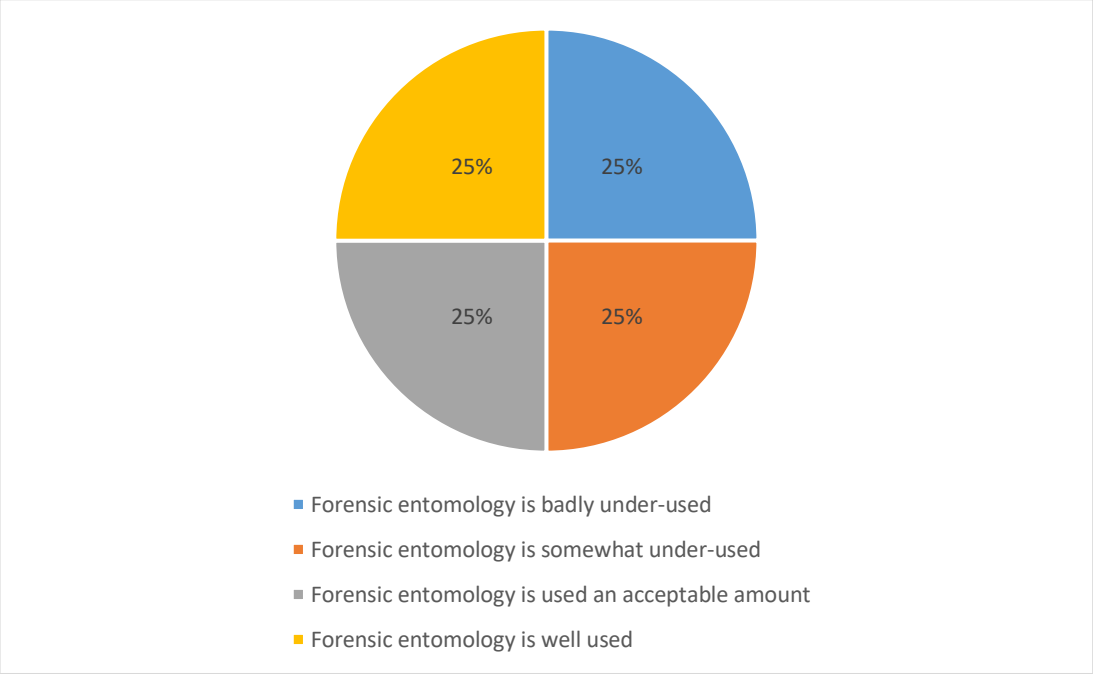

Figure 11: outlines the forensic entomologists' opinion on whether entomology is used to its full potential.

3.6.3 Findings from the Semi-Structured Interviews

The interviews involved three senior investigators, all of which had extensive experience in crime scene coordination, managing criminal investigations and major

incident room protocols, all were located in one force on the south coast of England. The interviews aimed to contextualise the operational utilisation of entomology in immersion death investigation through the viewpoint of the investigation leads. The approach to questioning the participants was semi-structured, with the questioning strategy ordered in the following way: the experiences of the participants in relation to immersion death investigations, exploring the use of entomology and associated expertise; opinion on the use of forensic entomology in immersion death investigation; what did they see as the gaps in current practice, and where can research help in the future. The interview transcriptions were analysed thematically, looking for correlating themes relating to entomology, immersion deaths and the investigative response.

The Experiences of the Participants in Relation to Immersion Death Investigations

All participants had experience of investigating immersion deaths and using entomological evidence, however none could recollect using entomological evidence in water related death investigations. The majority of the immersion death scenes they had encountered were at sea, or on the coast. One participant observing a case where intelligence provided detail of a possible deposition site at sea and the use of specialists in oceanography who helped to locate the remains. The other participants recollected body parts washed up on a local beach. All noted observing faunal activity, but none used it in any immersion death cases, one participant highlighting:

"I know what can be achieved with entomology generally, the use of flies, maggots, so forth, and we used it on cases a few times, but I know little of what or where water based creatures can assist?...." (P1)

"we wouldn't use [entomology] routinely, unless there was a clear investigative benefit and priority… it needs to be seen in the context of the investigation and the requirements" (P3)

"..in one case [immersion death], there were loads of crabs and creatures on the remains, we recorded this activity, but it wasn't used in evidence…" (P2)

In the majority of cases, there is a multiagency response with police working with the coastguard, paramedics and fire and rescue. The principal requirement is saving and protecting life with the investigative function following these activities. However, preserving the remains and the evidence in and around them, all agreed that the standard of forensic awareness amongst all agencies had improved:

"…once upon a time, there was some issues with scenes being completely cleared, or clothing removed and discarded, but this is rare now, the general preservation of evidence by all parties is good, also the recording of actions has improved.." (P3)

"Yes, work with the other agencies is pretty good now, communication is much better, in some areas it can be improved, but on the whole it's much better, and it needs to be.." (P1)

"We have worked hard linking in to the other agencies, we have little or no issues with preservation, I have worked closely with the health practitioners for example, there was an issue once upon a time, but that's why we met with them, set suitable protocols and now each side is aware of what the other needs and we continue to engage with each other…" (P2)

All agreed that the emergency responders were conscious of the need to preserve evidence where possible, but the way they recorded their actions had improved along with communicating what they had done to the police investigators. This helped the investigator account for relevant actions by other agencies and they can focus on the most relevant evidence.

Following this, discussion moved to the general use of expertise with investigating teams more aware of the need to call in advice from specialists and other agencies when required. This is was especially relevant in some immersion death scenes:

"…we will call in any expertise we need when necessary, perhaps the biologist, or someone who can help with tidal patterns. Early doors, we will have an investigation strategy in place and call who we need…" (P1)

Following the comment P1 made regarding using expertise, we asked whether this meant attending the scene or a review of evidence and findings without attending the scene. Their response was generally both, depending on the circumstances:

"…if there's an investigative need and scene interpretation requirement we will call experts to the scene… often my team at the scene will recover and submit that evidence after review, and then engage with the scientist. I can't think when I have called a forensic entomologist to the scene per se, but we receive advice off the crime scene managers, phone calls with the scientists themselves, and the pathologist…" (P1)

All participants had experienced immersion death scenes, and, in land based scenes at least, had used entomological evidence. However, as observed in the questionnaires, scenes were often few, sporadic and disparate; sometimes they would have three in one week, or none that were suspicious for months. Some highlighted the impact of this on the investigators and some were concerned about the awareness and pressure on investigators dealing with immersion death scenes for the first time. This is addressed in the sections below.

Opinion on the Use of Forensic Entomology in Immersion Death Investigation

The initial response to forensic entomology was a general awareness, but all in agreement, that it is seldom used and often it is not a priority:

"if there is a strong strategic requirement to use insects, I will.." (P1)

"..it's a niche science, and most of the time we can get better estimations of time of death from witnesses or other sources…" (P3)

"it really isn't a priority for us and is more often than not low down the priority list.." (P2)

All agreed that entomological evidence is infrequently used, and when it is required, they contact the scientist as with any other evidence types. However, nothing is a priority until or unless it is required, therefore it was suggested that there may be level of de-skilling, or an attrition in knowledge regarding undertaking actions or activities once in two years. P2 swiftly rebutted any issues regarding the "de-skilling" of the CSI through non-familiarity with recovery of evidence not routinely encountered:

"We have links with a local entomologist, we have a protocol in place and all my CSIs receive the necessary training on how to recover insects. So if it's there, and it fits the strategy, we can and will recover it…"

All agreed that they have never called an entomologist to a scene, but have engaged with them following evidence recovery and dissemination. In regards to the quality of recovery, all stated they do not recall any adverse feedback from the entomologist regarding the quality of packaging, and P2 re-iterated that they are fully aware of CSIs working with unfamiliar evidence and are conscious of the risk posed by unfamiliarity with the techniques or mechanisms of recovery. The protocols and necessary training are in place to cover this, and all the Crime Scene Managers know they can contact any specialist when required. However, this may not be the case in all jurisdictions:

"…we have these protocols in place, but I know many other areas don't. The processes across the country are very ad hoc at the moment, we have done it because we have experienced the fall-out in the past…" (P2)

In addition, this does not consider any possible difference of opinion between police and forensic entomologists regarding quality of packaging. Forensic entomologists may not have the opportunity to share negative feedback with police, and this would

be an interesting point to follow up in subsequent studies. In fact, as seen in figure 10, most of the entomologists surveyed here rated the quality of samples received from third parties as 'adequate' with few specimens being relevant or specimens being largely collected or packaged incorrectly resulting in moderate damage to specimens and some specimens being unusable. While this was assessed through the questionnaire and there was therefore no opportunity for follow-up questions to be asked, it does not seem a ringing endorsement of the quality of samples received from third parties including the police, especially when none of the entomologists rated the quality as 'excellent' and only one rated the quality as 'good'. This suggests that there might be a difference in how entomologists and non-entomologists are perceiving the quality of samples, however more data would be required in order to assess this fully.

In regard to immersion deaths, all participants acknowledged the complexities involved, in all stages of the investigations. Sometimes the search and recovery can be difficult and complex, but they were all aware of how oceanography and local knowledge could assist. However, all stated that it is important to be fully aware of what science can do. There is a need to know 'best evidence' in relation to the investigation requirements:

"I think it's important for all the investigators to know what's possible [in regards to science], not just in immersion deaths, but in all types of investigation, but the key priority for me is knowing when it's the right time to use it…" (P3)

"in the more major cases, I am confident we will throw everything at it, all the resources and so on….. when there are the 50 / 50 investigations, e.g. it's not obviously suspicious, but could be, when it's called non-suspicious early on, then suddenly becomes suspicious later, that's the issue, that's where we need to assist, perhaps the more junior investigators…." (P1)

The general feeling amongst the senior investigators was an acknowledgement of where and when entomological evidence could be utilised, but it needs to be at the

right time and this is infrequent. Similarly, regarding immersion death scenes, though often complex and introduced different types of risks, the investigators worked collaboratively with other agencies to ensure life was preserved, and there is safe and effective evidence recovery procedures in place. However, one participant in particular (P3), was adamant that we need more research and more detail on what is possible, so we know what is available in different situations and improve the effectiveness of our evidence recovery strategies:

"in all areas of investigation, we need to be ahead of the game, regarding entomology and its use for immersion deaths, as you call it, we must work with the researchers, the more we know the better I would like to see researchers and practitioners working closer to make research more relevant to inform my investigators.." (P3)

The emphasis here was the need to hone research to applied issues, but more importantly, convey this in a usable and clear way that informs practitioners without "jargon, or complex language". Any recommendations, promotion of new thinking and techniques needs to be grounded and informative at a practical level. Despite many comments from the practitioners that entomology in particular was a low priority, all expressed a strong feeling that there will be times when it may be the only evidential source available and therefore of higher strategic importance to the investigation. This reflects previously published research, such as Magni, Guercini, Leighton and Dadour (2013) who highlight the 'continued support and acceptance by both academics and practitioners as they work alongside the police and legal authorities'.

The Gaps in Current Practice, and Where Research Can Help in the Future

All participants agreed with the comments by P3 concerning better utilisation of research knowledge to inform practice. All related to the effects of current operational pressures that had made the utilisation of the forensic sciences more reactive, based on priority, and not necessarily used proactively, or to its full potential. In this regard, P1 and P3 commented on the operational pressures:

"..as an SIO, I don't have the luxury of submitting all evidence, I have to be selective based on the prioritisation, based on my resources, on what is most strategically important. That's why the more research I can have access to that tells me what I can glean from underwater decomposition and the impact this has on the evidence there, the better…" (P1)

"..we are reactively driven, but I think we can still make more use of the forensic research for intelligence purposes. If we engaged more with Universities, with researchers, etc. we can use this information more proactively…. Setting up resources information repositories we can access easily and effectively.." (P3)

P2 and P3 in particular were keen to link the more science-based knowledge with the working practices of the investigators. They both emphasised the need for this knowledge to inform the decision-making at scenes, to build awareness and to instil an instinctive awareness of when and where to process evidence in response to the investigative requirement. Much of this comes from learning and development:

"I want my investigators to think broader, not just about the obvious evidence, but be aware of the implications and instinctively know what is needed and when. More importantly, it's making those decisions quicker and under pressure.." (P3)

"many of my CSIs have a good knowledge of the science, but they need to have a working knowledge taking in the considerations at the scene, we can't do everything, but we need to instinctively know what we need, incorporate that in our forensic strategy linking that to what the science can realistically tell us" (P2)

In regard to research, all made the point of grounding research in the reality of investigations and the operational framework. They made the point that experts in forensic entomology, and any other discipline need to be aware of the working constraints of the investigator. All participants commented on the need for a two-way, reciprocal communication, which informs the investigators of what the forensic

entomologist needs, but also, what the scientist needs enabling realistic perceptions of how investigations operate and what their needs are:

"…..research needs to be grounded in the practicalities of the investigation, we shouldn't forget the ultimate aim is to bring justice….. Often valuable research is buried in journals, or gathering dust on shelves, I don't have time to read journals and translate it into easier to understand language, communicate with me, tell me what the science can do, clearly.." (P1)

"…of course, any research that informs me of what the evidence possibilities are and introduces new thinking to help me and my investigators is vital, but it often doesn't get where it's needed" (P3)

"…I work closely with researchers and students at our local [University], so we are able to utilise workable and usable research to improve what we do, more of this is what's required really.." (P2)

In fact, this need for a two-way working relationship is highlighted by Magni, Guercini, Leighton and Dadour (2013), who note that those forensic entomologists lacking undergraduate or technical level scientific training may not demonstrate the same ability to interpret information from the literature as those people with formal postgraduate levels of education, and as such application will suffer. Therefore, an active dialogue between scientists with specific postgraduate training and a robust understanding of scientific literature, and those practitioners who may have a working knowledge but no formal scientific education is crucial.

In regard to the gaps in current practice, all participants acknowledged research in immersion death and entomology is important. However, this needs to be in a format that practitioners can understand and the communication network between forensic scientist and crime scene professionals needs to be more robust with closer partnerships. Although, with the current operational frameworks and the fact that immersion deaths and entomology is seen as low down the priority list, with areas

like sexual offences, knife crime and cyber or computing crime taking precedence. In addition, national forensic services in the UK and across the world are moving towards more accountability, competency and accreditation, which is demanding much of the practitioners' time.

3.7 Conclusions

The review of literature, the questionnaires and interviews show that further research is required to fill the gaps in understanding, regarding forensic entomology and immersion death investigations. Though there are useful studies and some findings relevant to decomposition factors and tidal patterns, there is a dearth of research available in many geographical locations and for many water types, and less still focused on underwater faunal activities across varying species. This correlates with a desire from practitioners for more information on what science can do and how it can assist the investigation more effectively. By improving the awareness of what is scientifically possible, the more informed the investigators' forensic strategy will be. Subsequently, though participants stated attendance at immersion death scenes was relatively rare and the use of entomological evidence was limited, they acknowledged the need for better synthesis of research to practice, and a need to improve the embedding of new thinking and approaches into training regimes for operational personnel.

The focus of new research should inform the awareness of the forensic community, establish the possibilities and limitations of the evidence available and provide a suitable overview of the sampling methodologies. Ideally this should culminate in a workable and deployable model to inform practitioners and scientists alike. This paper aimed to provide the foundations for further studies to support an up-to-date awareness of methods and appropriate strategies flexible to the differing underwater habitats and contexts of any given water-based environment. Detailed awareness of the nature of the water, temperature, currents, tides, chemical and physical properties, natural fauna (and flora) present, notwithstanding the intrinsic physical and chemical conditions of the remains themselves, make every scene and body decomposition profile different. It requires a contextually relevant and bespoke forensic recovery strategy to maximise the best possible evidence available in each scene. This is limited to some extent as there are some sporadic studies in certain geographical areas, and some focus on casework, but little meaningful study on faunal activity underwater in many of the water types in the UK. What is also of note, is that there are no other meaningful compendiums or collections of key factors,

bringing the research together. Without this body of research, we cannot demonstrate the importance of entomological or other evidence consideration at underwater death scenes.

The questionnaire outcomes demonstrated that over half of the 28 CSIs felt they had a low or only moderate awareness of entomology, and interview participants P3 and P1 identified the need for improved awareness amongst early in career investigators and CSIs. This was Linked to better training, and better sharing of information to inform practice. All participants interviewed bemoaned the lack of research and new findings getting to where they are required. They stipulated a need for a closer union between scientists, researchers, CSIs and investigators to ensure a reciprocal programme of research that is mutually beneficial. P3 stated a need for investigators to be aware of the scope of the science, but more importantly, have a better awareness of where and how it can be used bespoke to each investigation. Therefore, the array of information outlined in the review of literature, along with the gaps, needs to be reconciled with the CSIs, forensic practitioners and police investigators. The questionnaire also confirmed the relatively low attendance at scenes and use of entomology by the CSIs, on reflection this introduced the risk of 'skill fade' and a lack of general awareness amongst CSIs regarding what evidence is available in underwater death investigations and how entomological evidence can be best utilised. Though the notion of skill fade was rebuffed by P2, it remains a risk unless, as stated previously, the decisions being made regarding when and where entomological evidence is useful is reinforced and regularly updated through regular experiential training regimes and appropriate aide memoires.

The questionnaire outcomes also demonstrated that experience equated to a more positive self-reflection and more confidence in the practitioners' own knowledge regarding entomology. Forensic entomologists, overall, reported that when they get the entomological evidence sent to them, the quality is often poor. Therefore, the need for awareness works across both fields, in that the entomologists need to communicate the appropriate processes regularly through updates and better communication. Though the interview participants were strong in defending the

training and awareness procedures in place for all practitioners, it is variable and disparate, with some remaining unsure of the appropriate protocols, the CSI responses to the questionnaire confirmed this. Conversely, there is also a danger of investigators being over-confident in their existing knowledge, if the appropriate information is not available and they remain unaware of the most up-to-date methods.

The investigation leaders highlighted that they need their teams to identify investigative requirements more instinctively, then have a broader awareness of what the science can do. It is now reactive, sometimes slow, and there are errors in judgement at the early investigative stages stemming from little effort promoting a broader awareness of what is possible. This also includes thinking broader, not just concentrating on the obvious evidence. Therefore, better communication and better engagement with less experienced practitioners utilising appropriate methods to promote learning and development is key to taking this forward. The training required is less about the science and more to do with improving decision making under pressure and making informed decisions. A part of this includes improving the contact between the entomologist and police forensic practitioners and developing regular research updates to inform practice more explicitly.

The interview participant P3 was concerned by the reactive use of forensic evidence generally and highlighted the lack of its utilisation for intelligence. Access to a databank of information to be available earlier to inform the investigation, for example, tides, currents, relevant models of processes, perhaps extending to entomological activity, known colonisations in different conditions, would be useful. The information may already be there, in various forms, but it is not easily available, nor is it in language or formats which can be easily understood. This is not to replace the expert, but to improve evidence capture and quality from the scene. The CSIs have collected insects at the scene, and more often than not, have not called the forensic entomologists, therefore the more the CSI can be educated and linked-in with what the science can tell the investigation, the more effective the forensic entomologist will be.

Priorities will change depending on the circumstances, and forensic practice is often vulnerable to responding to certain trends, therefore prioritisation and strategies will change according to demand. Research needs to be sympathetic with these trends and be available when required to inform frontline practice. The scientist needs to be more proactive in providing information to practitioners and reflect on the scene constraints, along with the organisation pressures, which impact on this practice. Much has been said about the prioritisation of the investigation, but more reflection on what is needed in practice, according to trends, is required to ensure research is relevant and applied to the needs of investigation and justice.

This paper has provided a foundation to inform further research by linking local law enforcement and national forensic entomologists to identify gaps and support future research in underwater forensic entomology. To the authors' knowledge it is the first time a detailed literature review, linked to practitioners and senior investigators' responses on the use of entomology and immersion deaths has been undertaken. It is clear that there is a lack of detail in the literature and a variance in practitioner awareness and deployment of entomology in the aquatic context. It demonstrates that research and capability is one thing, but this needs to be associated with practice constraints, and reliable communications networks. Underwater entomology has significant potential in certain circumstances, but the fostering of links and partnerships with the end-user should be an associated priority.

4. *Influence of Two Enclosed Water Types on Entomological Species Colonisation in Portsmouth, UK*

4.1 Statement

Article title: Influence of two enclosed water types on entomological species colonisation in Portsmouth, UK

Authorship details: Ody, H., Smith, P., and Brown, K.

Publication outlet: The British Journal of Entomology and Natural History

Current status: In preparation

The research for this paper was carried out in its entirety by the candidate, with support from the Institute of Criminal Justice Studies technical team. The article has been written in its entirety by the candidate, with support and feedback from Dr. Katherine Brown and Dr. Paul Smith. Revisions to this article have been made based on reviewer comments from previous submitted versions. A previous version of this article was published in the Portsmouth Postgraduate Review.

4.2 Abstract

The entomological species infesting rabbit *Oryctolague cuniculus* Linnaeus (Lagomorpha: Leporidae) carcasses decomposing in two enclosed water systems in Portsmouth, UK, were studied over a three-month period. The simulated water environments contained freshwater and sediment from a stream, and, the other, seawater and sand. Entomological samples were taken throughout the decomposition process until the carcasses were both fully disarticulated (151 days). A record of insect succession on the carcasses over time was produced and observations were made on the species, oviposition and development related to the decomposition process occurring in each environment. Although the carcass in the seawater initially appeared to be decomposing faster, both carcasses were in approximately the same state of decomposition by the end of the experimental period. There are currently no data on comparative colonisation for the South of England in aquatic environments. This study, which is part of broader research in aquatic death scene investigations, is focusing on the insect life in these

environments to inform investigators. The initial findings provide guidance for the collection of entomological specimens from remains found in small self-contained aquatic environments, an area that remains under-researched.

KEYWORDS Underwater, forensic entomology, insect succession, crime scene investigation, forensic science, PMSI

4.3. Introduction

4.3.1. Forensic Entomology and Aquatic Death Scenes

The study of insects and other arthropods for the estimation of minimum time since death (post-mortem interval; PMI_{min}) is well established. However, the study of insects in an underwater context is less developed. In aquatic death scene investigation, the study of insect life can help determine an estimate of minimum post-mortem submersion interval (PMSI). One of the main applications of forensic entomology in general is the estimation of minimum post-mortem interval (PMI_{min}), which is the amount of time elapsed between the death of the individual and discovery of the body (Amendt et al., 2007). This can help investigators to establish a timeline of events and help narrow focus on evidence relating to the peri-mortem period as well as eliminating erroneous leads. There are various different ways to estimate PMI_{min} including pathological methods (Goff, 2009) and mathematical models (Campobasso et al., 2001). However, the study of insect life present on remains is often used as an indicator due to the large amount of information which this can provide thereby increasing precision of PMI_{min} estimates (Amendt et al., 2004). These different methods are used in tandem to inform investigations with evidence coming from a range of different sources both investigative and scientific in nature.

The forensic entomologist can employ several different methods for estimating PMI_{min} from insect evidence. As blow flies are the most common initial colonizers of remains, they are often studied in great detail and can often provide the most useful indication of PMI_{min} (Amendt et al., 2004; Anderson, 2001; Greenberg, 1991; Hewadikaram & Goff, 1991). For example, the duration of larval growth can be used

to provide an estimate of time since death (Campobasso et al., 2001). Another method is the thermal summation model which uses flies' poikilothermic nature; the linear relationship between temperature and blow fly growth rates (Higley & Haskell, 2009). Faunal succession – the order in which insects colonize a body – can be used to estimate PMI_{min} throughout the decomposition period, especially in the later stages (Amendt et al., 2004; Anderson, 2001b; de Carvalho & Linhares, 2001; Greenberg, 1991; Hewadikaram & Goff, 1991; Higley & Haskell, 2009). This is because the order in which insects appear on remains is generally considered to form a recognisable sequence tied to the stages of decomposition with primary colonizers such as blow flies (Diptera: Calliphoridae) being followed by several species of beetle (Coleoptera) and to some extent some species of moths (Lepidoptera), wasps (Hymenoptera), arachnids (Arachnida) and roundworms (Nematoda) (Amendt et al., 2004; Campobasso et al., 2001). Some of these invertebrates are necrophagous, predatory or parasitic while others use the remains as part of their living environment (Amendt et al., 2004). Insect succession is also known to vary according to geographic location (Amendt et al., 2004).

While decomposition and insect succession on land has been studied in detail, the taphonomic processes and in particular insect succession underwater are not well documented. This is despite it being common for human remains to be found in water, with deaths resulting from accidents during recreation and disposal of remains following murders (Anderson, 2001). Suicide by drowning also occurs, especially in areas with easy access to water (Wirthwein, Barnard, & Prahlow, 2002). According to the Royal Society for the Prevention of Accidents (ROSPA) (ROSPA, 2014), in 2013 there were 381 drownings and water related deaths in the UK, of which 227 occurred in inland waters, and 137 occurred in or around coastal waters. In 2015, the number of people killed or saved in "near-fatal" accidents off the UK coastline was the highest since records began in 2011 (BBC News: UK, 2016). In more recent years there appears to be a trend towards reduced numbers of drowning deaths, however the number of these deaths is still not insignificant with 263 people losing their lives in accidental drownings in the UK in 2018 (ROSPA, 2019) This indicates a need for scientific research to aid in investigations and body recovery.

Whilst faunal succession in water is known to be different to that on land (Anderson, 2001), few studies have attempted to quantify the difference. In addition, the stages of decomposition themselves have been shown to differ between land and water. Payne (1965) described six stages of decomposition on land consisting of fresh, bloated, active decay, advanced decay, dry, and remains. Other authors have described variations on these stages, sometimes not distinguishing between active decay and advanced decay, leaving out the bloat stage, or leaving out the remains stage (Anderson & VanLaerhoven, 1996a; Reed, 1958; Rodriguez, 1982). In comparison, the stages of decomposition in water were first described by Payne and King (1972) as submerged fresh, early floating, floating decay, bloated deterioration, floating remains, and sunken remains. More recently, Hobischak and Anderson (2002) and Haefner et al. (2004) redefined the stages of decomposition in water as submerged fresh, early floating, floating decay, advanced floating decay, and sunken remains and these stages have been observed in case studies (for example Heaton, Lagden, Moffatt, & Simmons (2010)).

These stages of decomposition in water can help investigators provide an estimate of PMI_{min}, or as is more likely in an aquatic environment, post-mortem submersion interval (PMSI). In many cases PMSI, PMI and floating time of a corpse in water can be difficult to determine (Magni et al., 2014), and some crossover may exist between the terms. However as with PMI on land, it is possible to estimate PMSI based on insect evidence, although the factors which affect insect colonization of remains in water and on land are different. One such factor is the sink/float sequence which can be observed as bodies may float or sink upon entry to the water, remain submerged for up to three weeks in cold water (Magni et al., 2014), and then float again as gases build up during the bloated or early floating stages (Haefner et al., 2004). While the body is floating, there is the potential for it to be colonized by terrestrial insects including the blow flies which are so often used as early indicators of PMI_{min} on land (MacDonell & Anderson, 1997). Therefore, it is necessary to understand the interplay of these many different factors including the stages of decomposition in water, the characteristics of the water itself, including temperature, and the behaviour and

lifecycles of both terrestrial and aquatic invertebrates in order to successfully estimate PMSI.

Due to the unique circumstances surrounding forensic investigation in aquatic environments, well-established methodologies for terrestrial searches for people or objects are often not followed (Ruffell, Pringle, Cassella, Morgan, Ferguson, Heaton, Hope, & McKinley, 2017) and sometimes alternative or unconventional methods are required (Lew et al., 1996). Despite this, there is a requirement for investigators to work to specific standards, either due to ISO/IEC accreditation (specifically ISO 17020) or because of SOPs (Standard Operating Procedures) which set out a framework that must be followed for particular tasks.

4.3.2 Rationale

This study aims to provide insect succession data in different aquatic habitats, freshwater and seawater, in Portsmouth, Hampshire, UK. This is a coastal region with easy access to water, therefore it is important for practitioners to understand the taphonomic processes and be aware of the invertebrate species which may be important indicators and predictors in underwater death scene investigations. Since there is little data in this regard to inform the practitioner, especially location-specific data for the Portsmouth region, it is necessary to undertake the research to fill this knowledge gap and provide important data on the crime scene response to decomposed submerged remains (Chapter 5, unpublished data), along with careful and systematic recovery and treatment of entomological evidence from these environments.

4.4 Materials and Methods

In February 2016 two experiments were set up in Ravelin Park, Portsmouth, Hampshire (50°47'32.2"N 1°05'47.2"W) – an urban garden environment. The experiments were set up in translucent plastic boxes (26.5 x 15 x 17 cm), one per aquatic environment, with holes drilled in the lids to allow access by insects and rainwater while preventing access by larger scavengers such as foxes and seagulls

(Fig. 12). One box was filled with freshwater and a layer of sediment from a local stream (50°52'40.6"N 1°00'49.2"W) while the other was filled with seawater and a layer of sand from the sea at the south end of Portsea (50°46'41.5"N 1°05'04.2"W). This sediment/sand was included in case of any aquatic insects already being present. The boxes were placed in two areas of the garden which were as similar as possible to each other, however the box with freshwater was slightly more exposed and in a sunnier location while the seawater box was more shaded.

Figure 12: Plastic boxes with holes drilled in the lids to allow insect access were filled with water and sediment & water and sand, and placed into the garden environment.

A frozen-thawed eviscerated rabbit carcass, purchased as pet food, was placed into each box (Fig. 13).

Figure 13: Rabbit carcasses were paced into the plastic boxes

An infrared thermometer was used to monitor water temperature and temperatures were recorded daily except when sampling was undertaken by a Masters student on behalf of the researcher (see Table 1). The carcasses were then monitored and samples taken daily for the first 18 days until the carcasses were no longer visibly decomposing at such a fast rate, then every other day until the carcass sank below the surface of the water. From then, the carcasses were inspected visually every one to two weeks with samples taken whenever possible (i.e. whenever adult flies were present) until algal growth and products of decomposition prevented observation (day 78) (see Table 1). At this point, the rabbits were removed from the water once a month for three months (until 1st July 2016) in order to take samples and record the decomposition state (Fig. 14). Removal of the carcasses was conducted using a large mesh butterfly net with very fine holes (Fig. 15), which allowed the water to drain through whilst retaining any skeletal matter, tissue, or insects associated with the carcass.

Figure 14: The rabbits were removed from the water once a month in order to collect any insect specimens from the remains and record the decomposition state

Figure 15: Remains were removed from the water using a butterfly net

Samples in each case consisted of insect samples from the surface of the carcass, the water, and the air surrounding the boxes. Insect samples were primarily collected using forceps (for eggs, larvae, and dead adults from the surface of the water) and vial trapping (for live adults from the surface of the carcass) (Figs. 16 and 17).

Figure 16: Live adult flies were collected using vial trapping. Eggs were recovered using forceps.

Figure 17: Dead adult flies were recovered from the surface of the water.

Samples were preserved as per Amendt *et al*. (2004). Water temperatures were also taken using an infrared thermometer and photographs were taken to record the state of decomposition. In July 2016 after five months (151 days), the carcasses were fully disarticulated and were removed entirely from the water to be discarded.

Following this, the insect samples were identified in the laboratory using stereomicroscopic analysis of morphology (Ball, 2008; Grzywacz, n.d.; Skidmore, 1985).

4.4.1 Limitations

A primary limitation of this method is lack of replication. In this case the primary purpose of this study was to inform the researcher of insect species which may be found in a later field study, and results are not generalised beyond this. A full discussion of replication across all studies presented in this thesis is provided in Chapter 8, section 8.3.2: Replication and Pseudoreplication.

4.5 Results

Throughout the study, the water temperatures taken on site using an infrared thermometer remained fairly consistent with few large dips or spikes (Table 1). The temperature range for the seawater environment was 3.9^{o}C-15.1^{o}C and the range for the fresh water environment was 4.3^{o}C-14.9^{o}C. The temperatures were not taken at the same time each day, however the in each case the temperature was taken during daylight hours, between 0930 and 1625. Heavy rain was recorded on days 27 and 37, and samples were not taken on these days due to lack of insect activity.

Table 1: Water temperatures taken on site using an infrared thermometer

Day	Date	Time	Seawater Temperature (°C)	Fresh Water Temperature (°C)
0	01/02/16	1021	10.5	9.6
1	02/02/16	1053	10.8	10.6
2	03/02/16	1230	8.3	9.9
4	05/02/16	N/A	11.3	11.6
5	06/02/16	N/A	11.8	11.8
6	07/02/16	N/A	10.5	10.5
7	08/02/16	1221	8.4	9.6
8	09/02/16	1015	8.1	8.1
9	10/02/16	1000	7.1	7.1
10	11/02/16	1040	6.7	4.4
11	12/02/16	1005	7.5	7.7
12	13/02/16	1045	7.2	6.9
13	14/02/16	1055	6.7	7.3
14	15/02/16	1137	5.7	5.6
15	16/02/16	1035	5.3	5.5
16	17/02/16	0932	5.3	6.5
17	18/02/16	1408	6.7	7.2
18	19/02/16	0953	3.9	4.3
21	22/02/16	0945	10.0	9.4
23	24/02/16	1420	8.1	9.4
25	26/02/16	1340	7.2	8.1
29	01/03/16	1425	10.1	10.1
31	03/03/16	0930	7.4	N/A
35	07/03/16	1416	6.8	9.5
39	11/03/16	1625	7.9	N/A
41	13/03/16	1450	8.8	N/A
49	21/03/16	1408	12.6	12.9
78	19/04/16	1510	15.1	14.9
85	26/04/16	1430	12.9	12.9
120	31/05/16	1320	13.6	14.6

The dominant insect species encountered in both the seawater and freshwater environments was the blow fly *Calliphora vicina* (Robineau-Desvoidy, 1830), which appeared throughout the decomposition period (days 1-85). Appearances of another blow fly species, *Calliphora vomitoria*, were limited to the early post-mortem period. Other insect species including muscid and fannid flies appeared sporadically throughout, and a number of parasitic wasp specimens (Hymenoptera: Vespidae) were collected towards the end of the decomposition period. Tables 2 and 3 show a complete overview of insect species collected in both environments throughout the decomposition period (days 1-151). No insect specimens were collected after day 85 in the sea water environment as none were present on the final sampling occasion

(day 151). A graph showing frequency of species collected in the sea water environment is presented in figure 18. This is not representative of total numbers of flies present as not all flies were collected each time. Instead, an appropriate sample was collected in order to have an overview of the species present on each occasion.

Table 2: Overview of all specimens collected in sea water environment

Species	**Number of specimens collected per day**																												
Calliphora vicina	2	3	13	9	1	17	4	3	3	8	2	2	6	5	1	3	3	3	4	3	3	2	3	1	1	2	2	2
Calliphora vomitoria			1	1				1																				2
Phaeonia subventa					2						1							2	1				1	2				1
Phaeonia tuguriorum																			1									
Fanniidae sp.																												1
Fannia sp.										1																		
Fannia scalaris																			1									
Phoridae sp.															1													
Icneumonidae sp.																						1						
Alysiinae sp.																										1		
Sepsidae sp.																									2	2		
Cicadomorpha*									1																			
Unknown*																					2	1			1	1	1	6
DAY	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**14**	**15**	**16**	**17**	**18**	**21**	**23**	**25**	**29**	**31**	**35**	**39**	**41**	**49**	**78**	**85**

Table 3: Overview of all specimens collected in stream water environment

Species	Number of specimens collected per day																								
Calliphora vicina	1	2	10	6	4	5		1	4	22	3	5	9	6	5	3	4	2	3	3	3	2	1	2	
Calliphora vomitoria		1	1																						
Phaonia subventa										1											2	1			
Fanniidae sp.																						2			
Fannia sp.									1												1				
Anthiomyiidae sp.						1																			
Trichocera annulata							1												1						
Phoridae sp.											1														
Psychoda sp.											1														
Psychoda trinodulosa							1																		
Psychoda cinerea																									1
Alysiinae sp.																							1		
Aleocharinae sp.																									1
Syrphidae sp. (larvae)																									2
*Porcellio scaber**																								1	
Unknown*	2																	1	3						
DAY	**1**	**2**	**3**	**4**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**21**	**23**	**25**	**29**	**35**	**49**	**85**	**151**

Please note that the numbers of specimens in tables 1 and 2 only refer to amount collected and are not representative of overall abundance. Blank spaces indicate that no specimens of that particular species were collected on the corresponding day.

Carcasses were placed into the water on Day 0.

***denotes non-forensic (incidental) species or specimens which were too degraded/damaged to identify**

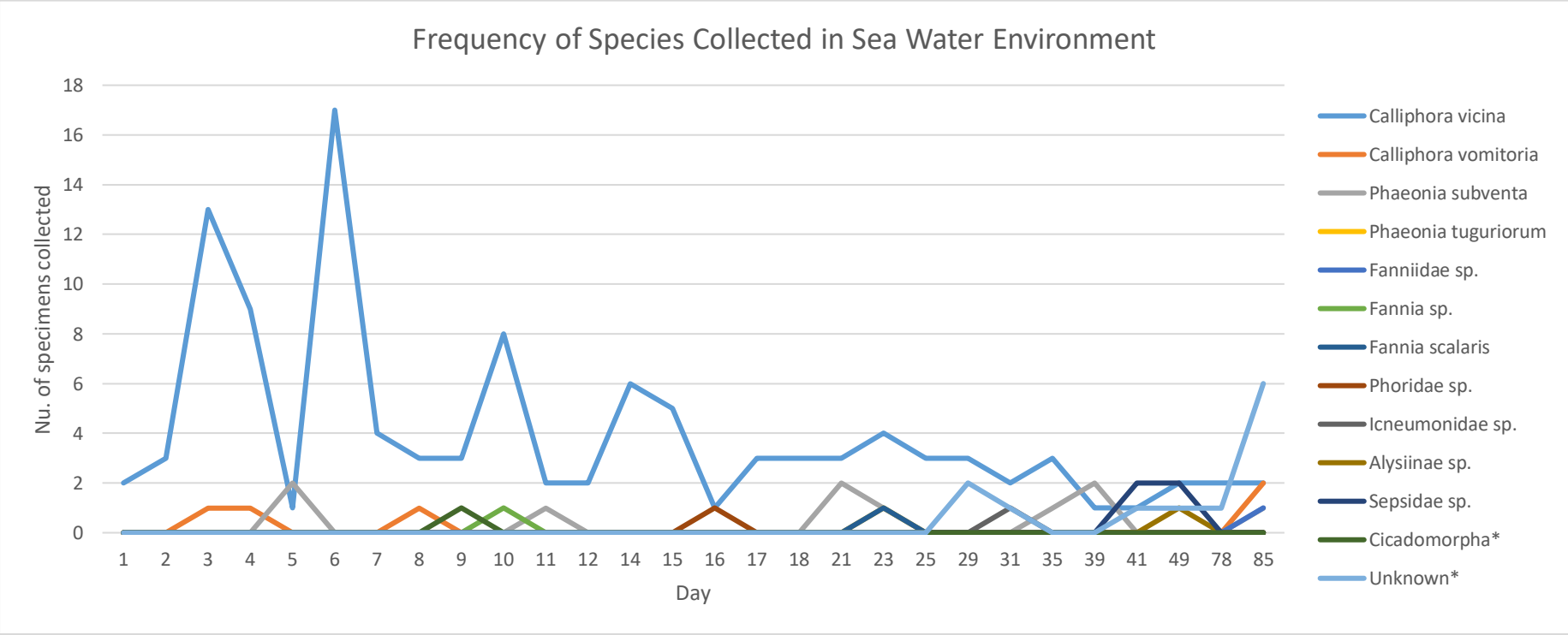

Figure 18: Frequency of insect species collected in the sea water environment.

The state of decomposition of the carcasses in both environments was compared throughout the experimental period (Table 4). The carcass placed into the seawater initially appeared to be decomposing at a faster rate than the one in freshwater, with visible decay appearing on the head at an early stage (day 8, Fig. 19, table 4). The carcass placed into the fresh water sank several weeks before the other (day 21 vs day 49, table 4). An overview of decomposition of the two carcasses can be seen in figures 19-26.

Figure 19: Visible decomposition appeared on the head (with eye and ear located in lower left corner) of the carcass in salt water early in the decomposition period.

Figure 20: The freshwater rabbit (left) and seawater rabbit (right) immediately after being placed into the tanks.

Figure 21: First appearance of visible decomposition (minor) in the freshwater rabbit (left) and the seawater rabbit (right). See table 4 for additional detail.

Figure 22: Blow fly activity on the seawater carcass, day 12

Figure 23: Increased decomposition on the head of the seawater rabbit, day 13

Figure 24: The freshwater rabbit remained partially submerged for several days

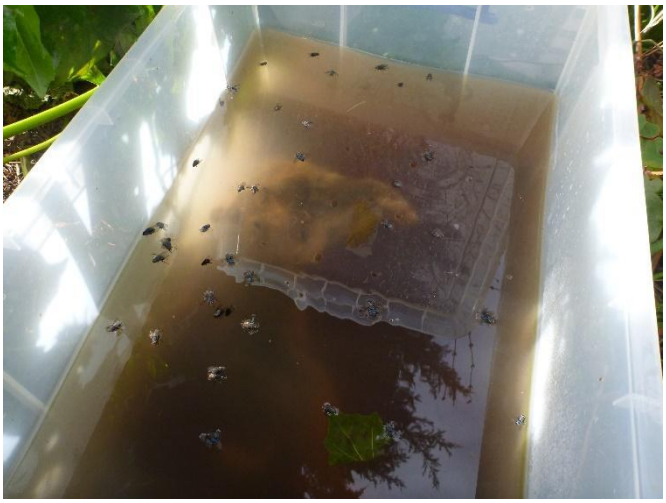

Figure 25: Adult blow flies were often observed having drowned in the water

Figure 26: The freshwater carcass at the end of the experiment. Right: the seawater carcass at the end of the experiment. At the end of the experimental period both carcasses were showing exposed bone with the head of the seawater rabbit being fully skeletonised

In this study, after approximately 78 days, it was no longer possible to see the carcasses through the water due to a combination of algal growth and discolouration of the water caused by the decomposition of the carcasses. Upon initial removal of the carcasses, it was found that the carcass placed into the salt water had fully skeletonised around the head, although no large larval mass was observed in this area prior to the carcass sinking.

Table 4: A comparison of the state of decomposition of carcasses in both environments over time.

Decomposition State	**Day first recorded - Freshwater**	**Day first recorded - Seawater**
Appearance of visible decomposition (minor)	10	8
Appearance of visible decomposition (significant)	N/A	12
Carcass beginning to sink	11	11
Carcass fully submerged	21	49
Carcass no longer visible through water (due to algal growth/decomposition)	78	78
Appearance of exposed bone	120	85
Carcass fully disarticulated	151	151

Note that samples were only taken daily for the first 18 days therefore in the later experimental period the day on which different states were first recorded only represents an approximation of when each state first appeared. N/A indicates carcass sank before any significant visible decomposition was recorded.

On 31st May 2016 (approximately four months after being placed into the water) the carcasses were removed from the water for inspection and it was found that both carcasses were in approximately the same state and were showing exposed bone in several areas.

By 1 July 2016 (day 151), the end of the study period, both carcasses were fully disarticulated. Although the carcasses had fully skeletonised, the remaining skeletal material was held together in a single mass by the remaining fur which had congealed but not fully decomposed.

Prior to the final removal of the carcasses from the boxes (1st July 2016), a thick grey-brown sludge had formed on the surface of the water. Upon closer inspection, this sludge appeared to be formed of a mixture of rabbit fur and tissue which had risen to the surface once the majority of the soft tissue had fully decomposed.

4.6 Discussion

4.6.1 Insect Colonisation and Sink/Float Sequence

The dominant species throughout the study, *Calliphora vicina,* is a species common throughout the UK in both urban and rural locations (Erzinçlioğlu, 1996) and routinely occurs as a primary coloniser of human remains. Other species which are known to be associated with human remains were found in both environments including several species of Muscidae and Fanniidae, notably *Phaonia subventa* (Harris, 1780) which have been recorded in other forensic entomology studies in England (Hwang & Turner, 2005) and Europe (Klimesova, Oleksakova, Bartak, & Sulakova, 2016; Prado e Castro, Serrano, Martins Da Silva, & Garcia, 2012). Like Calliphoridae, Muscidae are usually early colonisers of remains while Fanniidae appear slightly later on (Campobasso et al., 2001; Klimesova et al., 2016). This was the case in the seawater environment with Muscidae appearing earlier on (*Phaonia subventa* was first collected on day 5 while *Fannia sp.* was first collected on day 10), but this was not the case in the freshwater environment (where *Fannia sp.* was first collected on day 10 and *Phaonia subventa* was first collected on day 11).

Numbers of *C. vicina* peaked at day 3 and, in particular, day 11 in the fresh water environment. The peak at day 11 coincides with the appearance of visible decomposition which occurred during days 10 and 11 (see Table 4 above) however there was no obvious cause for the peak at day 3. In the seawater environment, *C. vicina* were most numerous on days 3 and 6, which also do not coincide with any of the observed decompositional changes noted in Table 4. There were no unusual dips or spikes in temperature which may have affected activity (Table 1). The differences may be due to chance or choice of sampling methods, and therefore it is necessary to repeat this study in order to gather enough data to verify whether these peaks are due to chance. Alternatively, there may be a change in VOCs which was not detected using the particular methods chosen for this study, but which could be detected by the sophisticated olfactory systems of the adult Calliphoridae (Frederickx et al., 2012).

Unusually for the area, no Luciliinae were recorded, however this was not the case in the subsequent freshwater study using piglet carcasses (see chapter 6).

Other species such as *Ichneumonidae sp.* and *Alysiinae sp.* were only recorded at the end of the experimental period, in keeping with previous research demonstrating that parasitoid wasps appear in the late postmortem period (Grassberger & Frank, 2003). Although the carcasses were eviscerated and therefore did not pass through the bloat phase (which often causes the carcass to re-float after initially sinking), there were a number of days after the carcass was first placed into the water and was still floating in which optimum conditions for colonisation by forensically relevant and other species were available.

In addition, the carcass in the freshwater sank several weeks before the one in seawater, which may have inhibited insect access and caused the remains to decompose at a slower rate. This is in line with other research demonstrating that remains decompose more slowly in sea water than in freshwater or on land due to reduced bacterial action due to salt concentration (De Donno et al., 2014). In contrast to this study in which both rabbit carcasses floated for some time after being placed into the water, Payne and King (1972), who decomposed piglet carcasses in metal tanks, record that most of their 11 piglet carcasses sank when placed into the water although a few remained floating. However, they do not state the source of the water or the weight of the piglets used in their study, nor do they record the amount of time it took for the piglet carcasses which initially floated to sink. Research indicates that remains with a higher body fat content are more likely to float (Schäfer, 1978 cited in Haglund and Sorg, 2002) while according to Magni *et al.* (2014) the initial sinking or floating of remains is affected by numerous factors, therefore it is not possible to make any generalisations and it is necessary to consider this on a case-by-case basis. This significantly affects the rate of decomposition and it must be taken into account that this variable is not predictable as of yet. Despite this, building up a databank of observations under controlled conditions is still important both for research and casework as investigations benefit from having detailed empirical knowledge to draw on.

The factors which influence the sink/float sequence may include water type, and this factor may also have an effect on the overall decomposition of the carcasses, as was the case in the initial stages of this study in which the freshwater carcass appeared to be decomposing at a faster rate. However, by the end of the experimental period, both carcasses were in approximately the same state. This suggests that after an extended period underwater in an enclosed environment, the type of water plays a limited role in the timing of decomposition. Similar findings are reported by Heaton et al. (2010), where bodies found in different rivers and canals in the UK all passed through the same stages of decomposition at the same time. However, since only freshwater environments were studied by these authors, it is not possible to draw a comparison with bodies found in seawater environments during the same time period.

The choice of carcass for this study must also be taken into account. The aim of this study was to investigate colonisation and, subsequently the carcass was merely used for this purpose, to monitor insect succession, and not, necessarily, as an analogue to extrapolate to human decomposition. It should be noted that, generally, domestic pigs (*Sus scrofa domesticus*) are considered the best carcass type for decomposition studies due to their similarities to humans in terms of factors such as omnivorous diet, fat distribution, and lack of heavy fur (Anderson & VanLaerhoven, 1996b; Byrd & Castner, 2009; Catts, 1992; Schoenly et al., 2006). Interestingly, the heavy fur present on the rabbit carcasses used during this study held the skeletonised remains together in a single mass even after the soft tissue had decomposed, however it is unlikely that this would occur in less heavily furred mammals if they are unclothed, including the pig carcasses, which are preferred for this type of research, and humans. While in some circumstances layers of clothing can hold skeletonised bodies together even in water (Dumser & Türkay, 2008; Lew et al., 1996) it has been observed, at least in some cases, that where clothed bodies have been found in water, the layers of clothing have prevented scavengers and insects from accessing the body and that only exposed parts have become skeletonised (De Donno et al., 2014; Introna et al., 2012). As such, disarticulated skeletal material may be held together by clothing in a similar manner and therefore the presence of clothing on a

submerged cadaver looks to be a significant factor in decomposition rate that requires further exploration. In addition, it is likely that most decedents in aquatic deaths are clothed, as is the case in Hampshire according to Hampshire Constabulary (pers. comm.).

According to Haynes (1980, cited in Haglund and Sorg, 2002) animal hair increases the buoyancy of carcasses as it traps air, and this may have impacted on the sink/float sequence of the carcasses used here as compared to human or pig carcasses. However, trapped air in clothing may cause the same effect (Spitz, 1980:360 cited in Haglund and Sorg, 2002). As previously noted, buoyancy of remains is a complicated issue involving many different factors and as such it is difficult to draw any conclusions about how much difference (if any) there might be between buoyancy caused by animal fur and buoyancy caused by air trapped in clothing.

4.6.2 Decomposition of Carcasses in Enclosed Aquatic Environments

Other studies have investigated decomposition of carcasses in enclosed systems, notably Payne and King (1972) who studied decomposition of piglet carcasses inside metal tanks. This method appears to be a less accurate predictor of the results in natural aquatic environments (Hobischak & Anderson, 2002), however the results of this type of study may be more applicable to decomposition in other enclosed systems for example a septic tank (such as that described by Lew, Bannach & Rodriguez (1996)). While the remains in this example were fully skeletonised by the time they were recovered, the methods used to recover the remains were comparable and included recovery of anything visible by hand and complete drainage of the tank to ensure as far as possible that all skeletal material and other evidence was recovered. In addition, the skeletonised remains were partially held together by clothing, with exposed bones being recovered either from the liquid within the tank, or floating in the semisolid layer of scum at the surface therefore indicating search locations for bodies recovered from water (Lew et al., 1996). Similar experimental setups have also been used to study adipocere formation, such as in the case of Stuart, Notter, Dent, Selvalatchmanan, & Fu (2015) who used sealed polyethylene containers filled with seawater collected from Sydney Harbour, river water collected

from a river in Goulburn, New South Wales, and chlorinated swimming pool water from a domestic outdoor swimming pool. Giancamillo et al. (2010) also used tanks to house piglets decomposing in fresh water, on which they performed microscopic and macroscopic taphonomic analysis as well as examination of brain, heart, and other tissue for the presence of diatoms from the water.

In the plastic boxes used to house the rabbit carcasses for this experiment, the holes drilled in the lids allowed access by the adult flies but prevented their escape. The resulting layer of dead adults on the surface of the water (Fig. 27) may have contributed to the discolouration of the water which after 78 days prevented observation of the carcasses without their removal. In addition, it is likely that this would not have occurred had the boxes been left uncovered and consequently fewer drowned adult flies may be found in more open water systems such as a pond or lake. As it was also not possible to tell exactly when during the decomposition period the adult flies had drowned, this may also have had a bearing on the pattern of insect succession recorded. This phenomenon may have had an effect on insect colonization of the remains, as it is possible that adult flies were drowning before having the opportunity to oviposit – but this would occur in any case if they were to enter the water and be unable to escape. This would also cause a slower rate of decay due to there being very low numbers of feeding larvae. These observations may be important in cases where bodies are found in enclosed systems like the remains found in the septic tank, however this is also different to larger ponds or flowing systems where access would be greater.

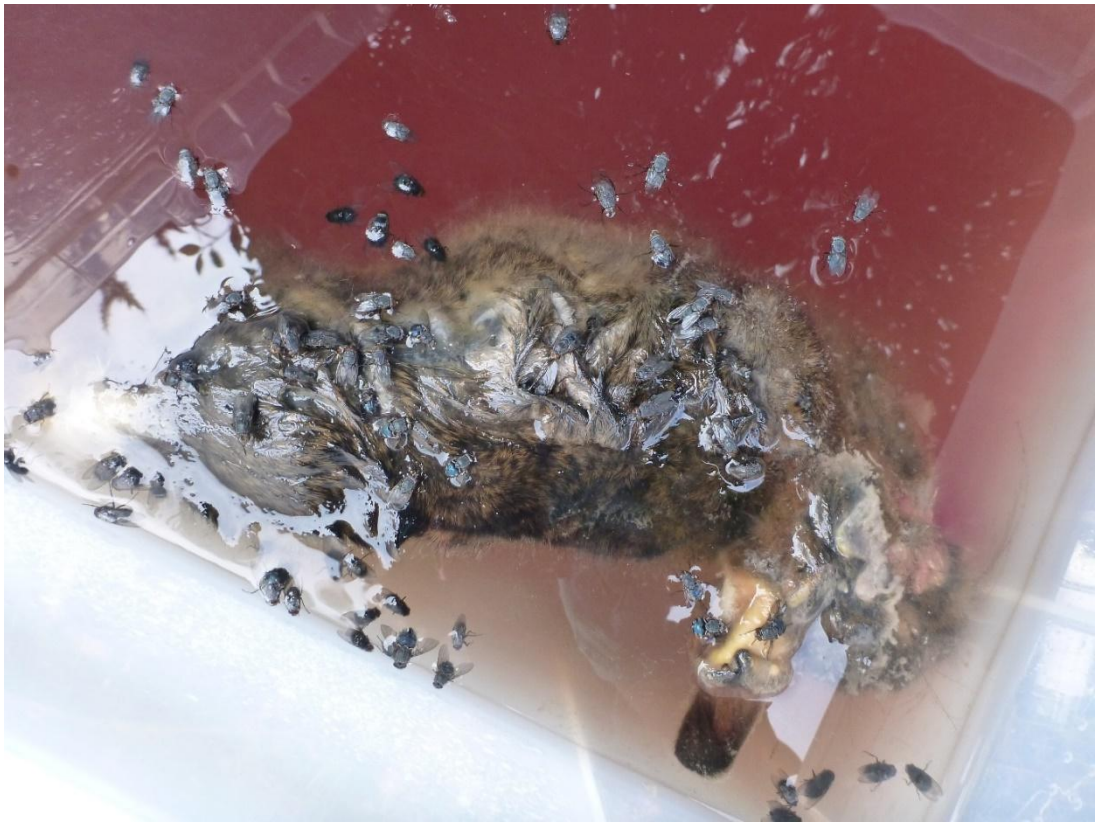

Figure 27: Many flies drowned in the tanks as they were able to enter through the holes in the lid but were unable to escape.

4.6.3 Approaches to Entomological Sample Collection

In the case of the skeletonised remains found in the septic tank, it was necessary to employ unconventional methods to facilitate the removal of the body, including use of a backhoe which would not normally be considered acceptable in most excavations (Lew et al., 1996). In the present study, it was also found that alternative approaches to collection and sampling were necessary, demonstrating that death scene investigations involving bodies in contained aquatic environments require additional thought and versatility according to different circumstances. However, this has the potential to be made more difficult by SOPs, such as those published by the College of Policing (ENSFI Scenes of Crime Working Group, 2012) and ISO/IEC 17020 under which forensic providers are held to rigid quality standards for their scene examination work (Science and Technology Committee, 2013). These exist to provide a standardised and systematic approach, which helps with demonstrating quality of service provision but may not leave room for investigators to employ unconventional methods of evidence recovery where circumstances require it.

Existing protocols published by the European Association for Forensic Entomology (EAFE) (Amendt et al., 2007) advise on types of equipment and methods for collection, including storage, of entomological evidence. However, some of these methods were found not to be useful for the circumstances of this particular study. For example, spoons are recommended for the collection of larvae however in this case it was necessary to pick up larvae individually from the surface of the carcass using forceps. The reason for this was twofold: firstly, the surface of the carcasses was wet from having been in the water and therefore the larvae tended to adhere to the surface such that they could not be lifted with a spoon without risking them becoming squashed, and secondly the larvae had become tangled or embedded in the fur of the rabbits again making lifting them with a spoon very difficult. Therefore, if collecting larvae from a human cadaver found in water, it may be necessary to adapt the collection methods to the situation either to avoid damaging specimens or for ease of collection where larvae may have become embedded in hair or clothing. As such, this requires researchers to work with police forces and CSIs to develop protocols and working relationships in order to get the best from the evidence.

For example, in the present study very few adult insects were trapped from the air above the carcasses as very few adult insects were observed flying above the carcasses throughout the decomposition period. This contrasts with other studies, particularly those investigating decomposition on land, where adult insects are commonly sampled using sweep netting. However, this is also size dependent with smaller carcasses (such as the rabbits used in this case) attracting fewer flies than larger ones (Simmons, Adlam, & Moffatt, 2010). In addition, although vial trapping was used to collect live adult blowflies from the surface of the carcasses in this case (as it can be from carcasses on land), it was found that care must be taken to avoid pushing the carcasses below the surface of the water and therefore potentially losing or disrupting other evidence. As such, if collection of entomological evidence is undertaken while the remains are still in the water, investigators should pay particular attention to the need to collect other types of forensic evidence which could be compromised by the choice of collection method for entomological evidence. However, this may be more relevant to forensic entomology research in

which researchers may wish to leave carcasses in the water than to case work in which remains are likely to be removed. To aid with improved collection of entomological evidence, additional training on alternative collection methods could be provided to personnel including CSIs, entomologists and pathologists.

Various types of evidence have been shown to be recoverable from submerged remains or other items including fingerprints, DNA from bite marks, explosive residues, blood evidence, hair and fibre evidence, and other trace evidence (Dutelle, 2007; Kamyshny, Magdassi, Avissar, & Almog, 2003). As some of these evidence types are transient and easily lost–for example hair and fibre evidence-it is of paramount importance to ensure that the collection of this type of evidence does not suffer as a result of the use of unsuitable collection methods for insect evidence. Continued training and dialogue with CSIs is imperative to ensure that correct protocols are produced and followed. This also applies to research in forensic entomology, where the accuracy of results may be compromised by the choice of sampling method.

4.7 Conclusion

Insect succession in water has been shown to differ according to geographical region, and although the processes of decomposition and insect succession are known to differ in water compared to on land, underwater decomposition has not been studied in great detail. In particular, no data is available from research studies in the United Kingdom, although some knowledge has been gained from case studies. In this study, it was possible to provide an overview of insect succession on rabbit cadavers decomposing in freshwater and in seawater in enclosed environments, considering the effect of the enclosed system on data collection (i.e. that insects may be able to enter the system but be unable to escape thereby drowning in the water, which in turn could cause confusion due to it not being immediately evident how long each specimen has been present). While this data is less applicable to decomposition occurring in more open aquatic habitats, it nonetheless provides baseline data for aquatic decomposition in the Portsmouth area, which can be drawn upon for future research in both enclosed and open aquatic environments. As this study included only one replicate in each environment, a further study is currently being undertaken

in more natural environments in order to verify the insect succession data, however the recommendations for sampling/data collection methods can be taken for use in further research. These recommendations may be especially applicable to certain circumstances especially where entomological evidence is being recovered by someone other than the forensic entomologist.

In many respects the use of entomological evidence in general is relatively under-used in case work. This study provides some initial data to inform practitioners regarding considerations for investigations relating to submerged remains. The research group are working collaboratively with police to identify where the information can be used to support relevant investigative strategies and provide additional intelligence for enhanced concordant thinking in death investigations.

5. A Preliminary Investigation of Faunal Colonisation of Remains in Open Water

5.1 Statement

Article title: A Preliminary Investigation of Faunal Colonisation of Remains in Open Water

Authorship details: Ody, H., Smith, P., and Brown, K.

Publication outlet: Forensic Science International (Technical Note)

Current status: In preparation

The research for this paper was carried out in its entirety by the candidate, with support from the Institute of Criminal Justice Studies technical team and the Institute of Marine Sciences technical team. The article has been written in its entirety by the candidate, with support and feedback from Dr Katherine Brown and Dr Paul Smith.

5.2 Abstract

Insect and invertebrate colonisation of remains in aquatic environments is a current topic of interest in forensic entomological research. Here, three approaches for investigating invertebrate colonisation on animal remains in open water were tested, with a view to providing the first steps towards a robust methodology for conducting this type of research as a stepping stone to a larger scale study. Method 1 used a modified crayfish pot to enclose a piglet carcass and incorporated a GoPro™ camera to monitor decomposition and marine fauna feeding behaviour using timelapse photography. Method 2 made use of a whelk pot to house the carcass and improve the rate of trapping feeding fauna. Finally, method 3 took a combined approach using a lobster pot to both house the piglet carcass and trap feeding fauna, as well as using timelapse photography to monitor decomposition and feeding behaviour. Of these, method 3 was the most successful for monitoring decomposition and colonisation for forensic purposes as it was possible to collect much better quality data using this method than with the other two.

KEYWORDS: forensic science; forensic entomology; aquatic forensic entomology

Invertebrate colonisation and decomposition of remains in aquatic environments is typically under-researched, however more consideration has been given to this topic in recent years (Barrios & Wolff, 2011; Celata, 2015; Chin, Marwi, Jeffrey, & Omar, 2008; Humphreys, Panacek, Green, & Albers, 2013; Pakosh & Rogers, 2009; Widya, Moffatt, & Simmons, 2012). Due to the numerous difficulties with researching invertebrate colonisation on remains in aquatic environments, particularly in open water, much of the existing research has been conducted either as case research (such as Ambade et al., 2013; De Donno et al., 2014; Dumser & Türkay, 2008; González Medina, Soriano Hernando, & Jiménez Ríos, 2015; Magni, Borrini, & Dadour, 2013; Magni, Pérez-Bañón, Borrini, & Dadour, 2013; Petrik, Hobischak, & Anderson, 2004; Wallace, Merritt, Kimbirauskas, Benbow, & McIntosh, 2008) or on a very large scale including the use of highly specialised equipment or divers which may not be available to many laboratories (Anderson & Bell, 2014, 2016, 2017; Anderson & Hobischak, 2004; Anderson, 2008), particularly in parts of the world where forensic entomology research faces barriers such as lack of cooperation between experts in different disciplines, police personnel, and CSIs (Amendt, Krettek, Niess, Zehner, & Bratzke, 2000; Gomes & Von Zuben, 2006) or insufficient funding (Vasconcelos & Araujo, 2012). However, it is common for human remains to be found in water whether through accidental means, body deposition following murder, or even suicide (Anderson, 2001b; Wirthwein et al., 2002), with 263 people drowning by accident in the UK in 2018 (ROSPA, 2019) and similar numbers in the previous two years (ROSPA, 2018). Therefore, in order to investigate aquatic death scenes, there exists a clear need for taphonomy and forensic entomology laboratories worldwide to be able to investigate the phenomenon. This is especially true because of the differences between different water types in terms of biodiversity, temperature, and chemistry, and therefore decomposition and insect succession in different aquatic environments must be explored fully. Here, three methods for researching invertebrate colonisation and decomposition of remains in sea water were tested, with a view to providing a robust methodology using inexpensive and readily available equipment.

5.3 Methods

All studies were conducted on a Marine Research Raft (Figures 28 and 29), using stillborn piglet (*Sus scrofa domesticus*) carcasses donated by a local pig farmer weighing between 0.05kg and 3kg.

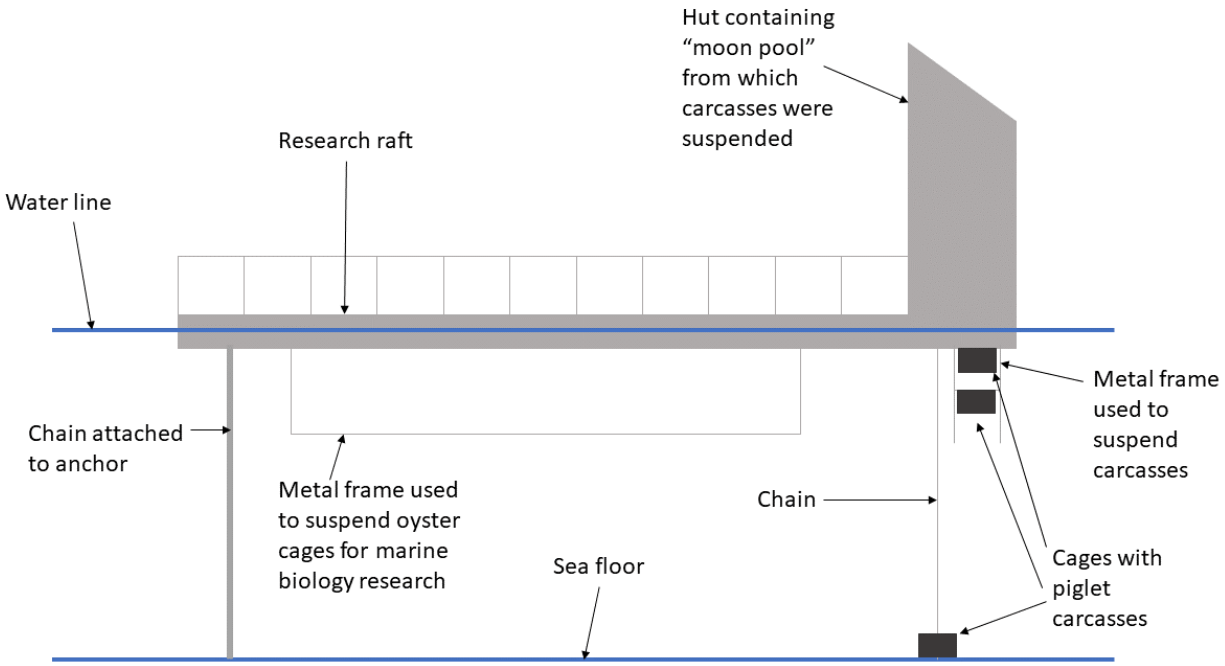

Figure 28: Diagram of the University of Portsmouth marine research raft set up for method 1a

The raft is located in Langstone Harbour, Portsmouth, UK (Figures 30 and 31) and is only accessible by private boat.

Figure 29: View of the University of Portsmouth Marine Research Raft showing the metal frames used to suspend oyster cages for marine biology research

Figure 30: Location of the University of Portsmouth Marine Research Raft in Langstone Harbour, indicated by the red circle (Google Maps, n.d.).

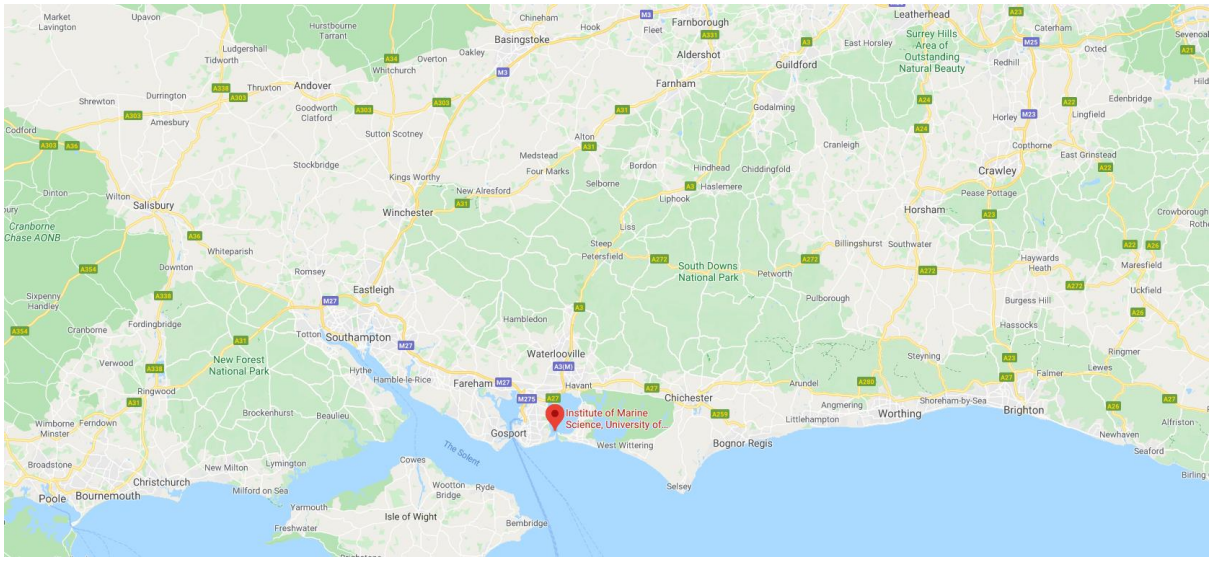

Figure 31: Map showing location of the University of Portsmouth Institute of Marine Sciences in Langstone Harbour - south coast of England (Google Maps, n.d.)

1a) Modified crayfish pot and 1b) Modified crayfish pot with GoPro™ camera

A plastic, barrel shaped crayfish pot was used to act as a container for a piglet carcass (Figure 32).

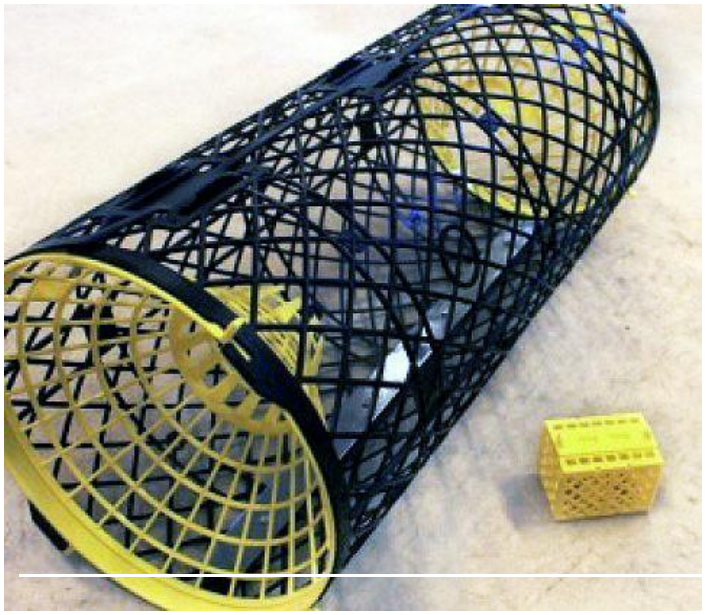

Figure 32: Barrel-shaped crayfish pot (image from https://store.coastalnets.co.uk/collections/pots/products/crayfish-pots)

The yellow funnel-shaped one-way entrances were removed from each end. A piglet carcass was placed inside the trap. The open ends of the remaining plastic mesh tube were then secured shut using zip ties, forming a “cage” to enclose the carcass within (figure 33).

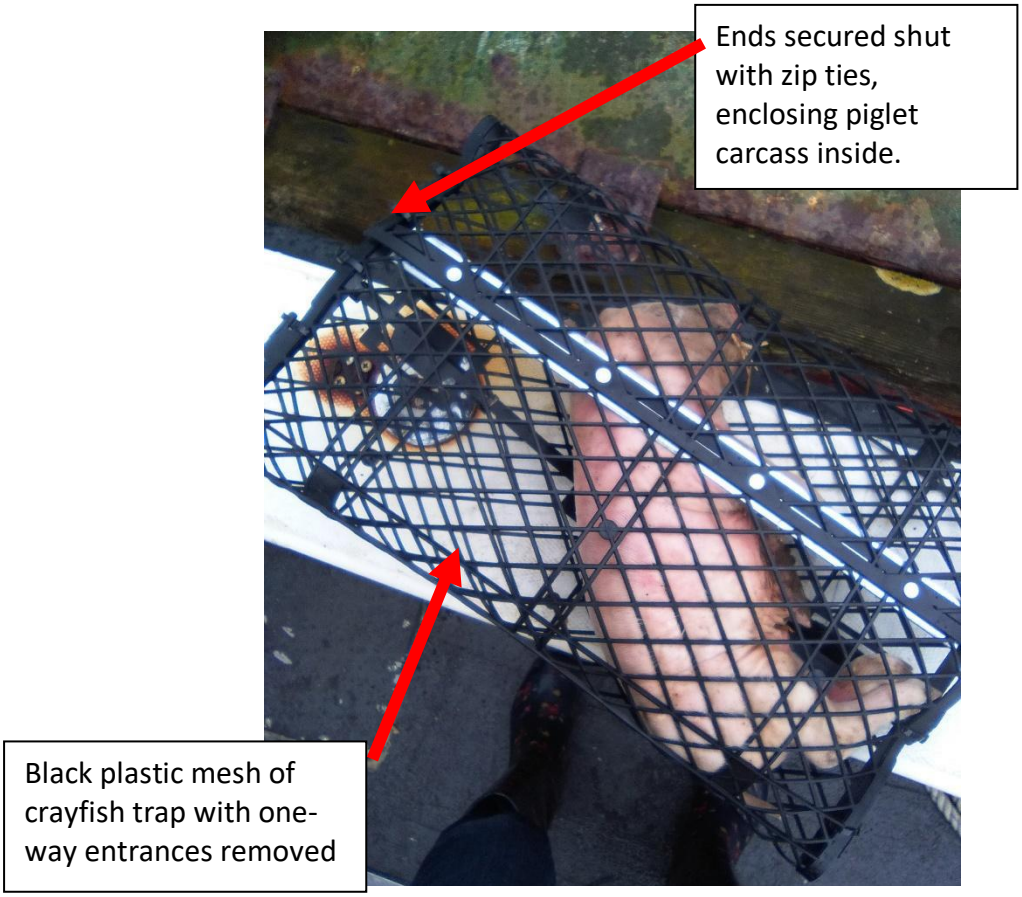

Figure 33: A piglet inside one of the modified crayfish traps

In the first instance, three such cages were prepared. Two were attached using zip ties to a metal frame (58.4 cm x 104.1 cm) which could then be suspended from the raft, submerging the lower of the two pigs pig by approximately 76 cm (figure 34). The third was attached to an anchor and lowered to the sea floor (4-9m depending on tides). These three depths were chosen to assess any differences in fauna appearing at different levels in the water column.

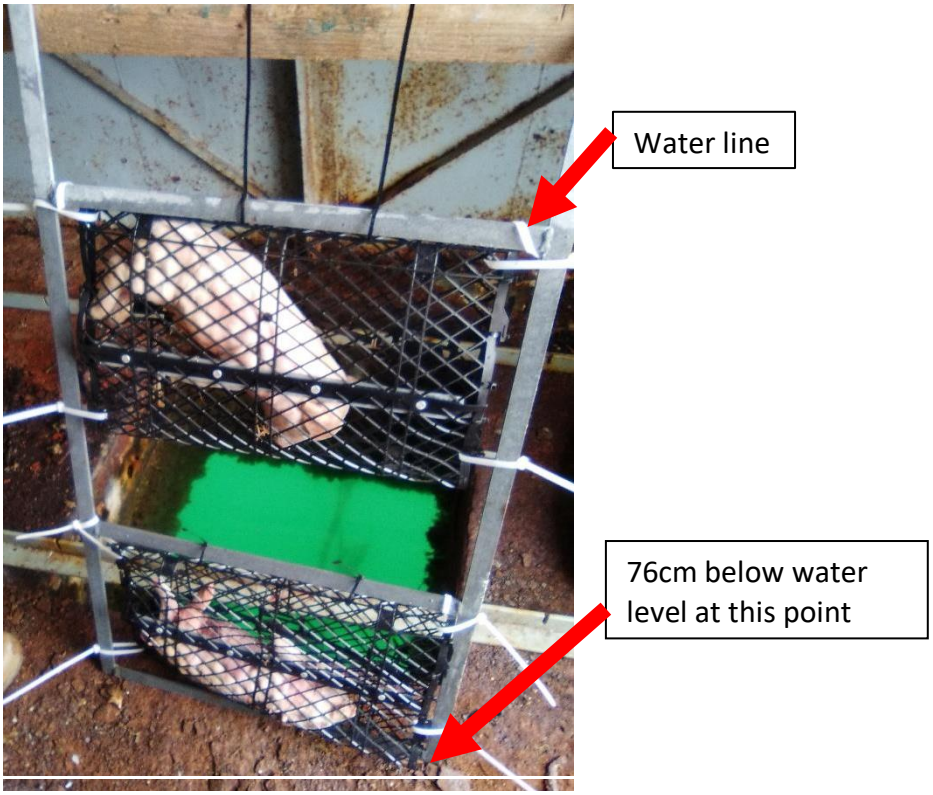

Figure 34: Two cages (modified crayfish pots) were secured using zip ties to a metal frame which was then suspended from the raft, submerging the lower pig by approximately 76cm.

The frame is shown in situ in figure 35.

Figure 35: The frame was suspended from the raft with the uppermost pig just under the water level and the bottom of the lower pig submerged by approx. 76cm.

An anchor was attached to one end of the third cage and a chain to the other, allowing it to be lowered to the sea floor (4-9m deep depending on tides) and attached to the raft to prevent loss of the equipment (figure 36).

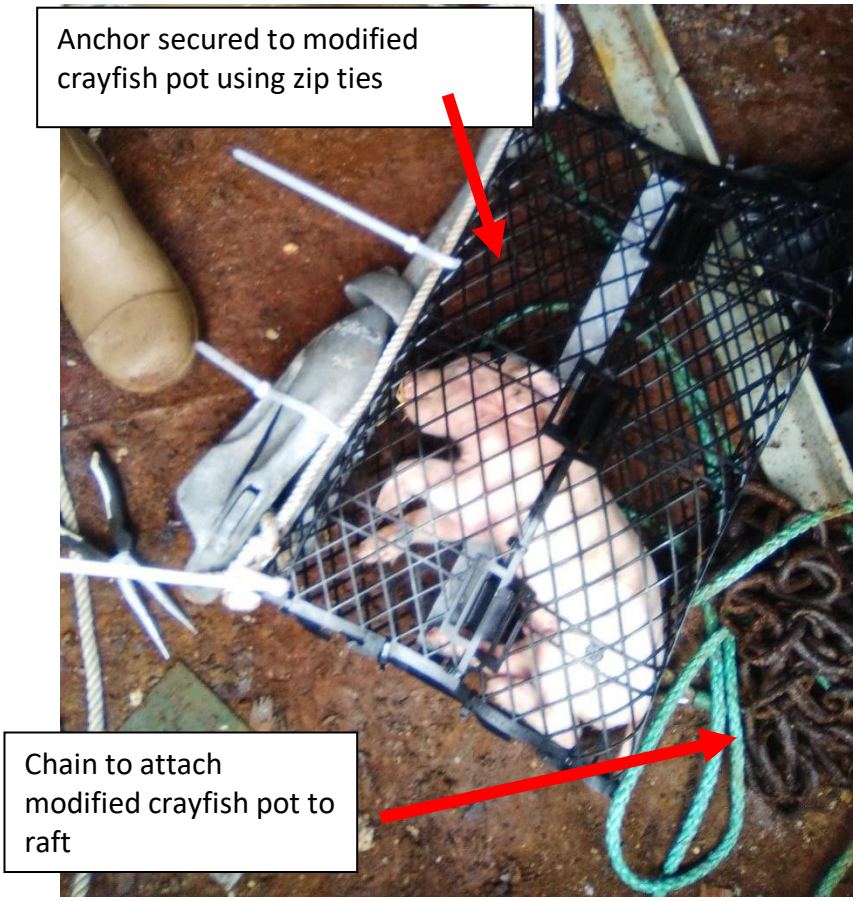

Figure 36: The third cage (modified crayfish pot) was attached to an anchor and lowered to the sea floor. A chain was used to secure the cage to the raft to prevent it being lost.

The mesh design of the cage allowed feeding by any invertebrates or other fauna, whilst preventing the piglet carcass from being removed as a whole.

The cages were checked approximately once a week (depending on availability of technical staff with the appropriate training and licensing to sail the boat) between 23rd June 2016 and 12th August 2016. This was done by lifting the metal frame out of the water and onto the raft, and pulling the third cage up from the sea floor using the chain.

A second trial using only the two cages attached to the metal frame was run (uppermost pig just under the water level and lower pig submerged by approx. 76cm), and the carcasses were checked approximately every two weeks between 10th November 2017 and 1st February 2017.

A follow-up experiment was undertaken in which the frame was removed from the setup and a GoPro Hero 4™ camera (https://gopro.com/) and Blink Time Lapse Controller™ (https://cam-do.com/products/blink-gopro-time-lapse-controller) with waterproof housing was added to the cage anchored to the sea floor in order to increase the amount of data it was possible to collect. Using the Blink attachment, still frame photographs were taken every 30 minutes, with the camera powering down in between shots to preserve the battery life. The experiment ran until the carcass was fully decomposed (25th July 2017 – 3rd August 2017). The cage was only checked at the end of the 10-day period, relying on camera footage to identify larger scavengers.

2) Whelk Pot

A standard 20 litre plastic whelk pot was trialled as an alternative trap design to assess whether the greater amount of space in between the carcass and the sides of the trap would be more successful at trapping feeding organisms (Figure 37). In this case, the piglet carcass was secured inside the pot using zip ties attached to the inbuilt wire hook (normally used for spearing bait). The pot was weighted with concrete (inbuilt), attached to the raft via a length of rope and a chain, and then lowered to the sea floor. To check the decomposition state of the carcass and record any colonisation of the remains, the pot was pulled up from the sea floor using the chain. The pot was checked approximately once every two weeks between 10th November 2017 and 1st February 2017.

Figure 37: A 20 litre plastic whelk pot containing a piglet carcass

3) Lobster Pot with GoPro™ Camera

A creel shaped lobster pot (https://store.coastalnets.co.uk/collections/fishing/products/creel-shape-lobster-pot) fitted with a GoPro Hero 4™ camera and Blink Time Lapse Controller™ was also trialled. Later, the inside of the lobster pot was lined along the bottom and sides with fine plastic mesh to help prevent loss of skeletal matter and smaller fauna from the trap (Figure 38). A chain was attached to the pot in order to secure it to the raft, and the pot was lowered to the sea floor. No additional weights were added to the pot, allowing it to rest vertically with the hinged narrow end resting on the sea floor. To examine the carcass (in addition to monitoring the GoPro™ images), the lobster pot was pulled up from the sea floor using the chain. The pot was placed into the water on Friday 26th January 2018 and removed from the water for the final time on 12th February 2018.

Due to the design of the lobster pot, it somewhat mitigated any potential problems with large predators eating the whole carcass as they were more likely to leave the kitchen portion of the pot and enter the parlour, from which they were then unable to return to the carcass (Figure 38).

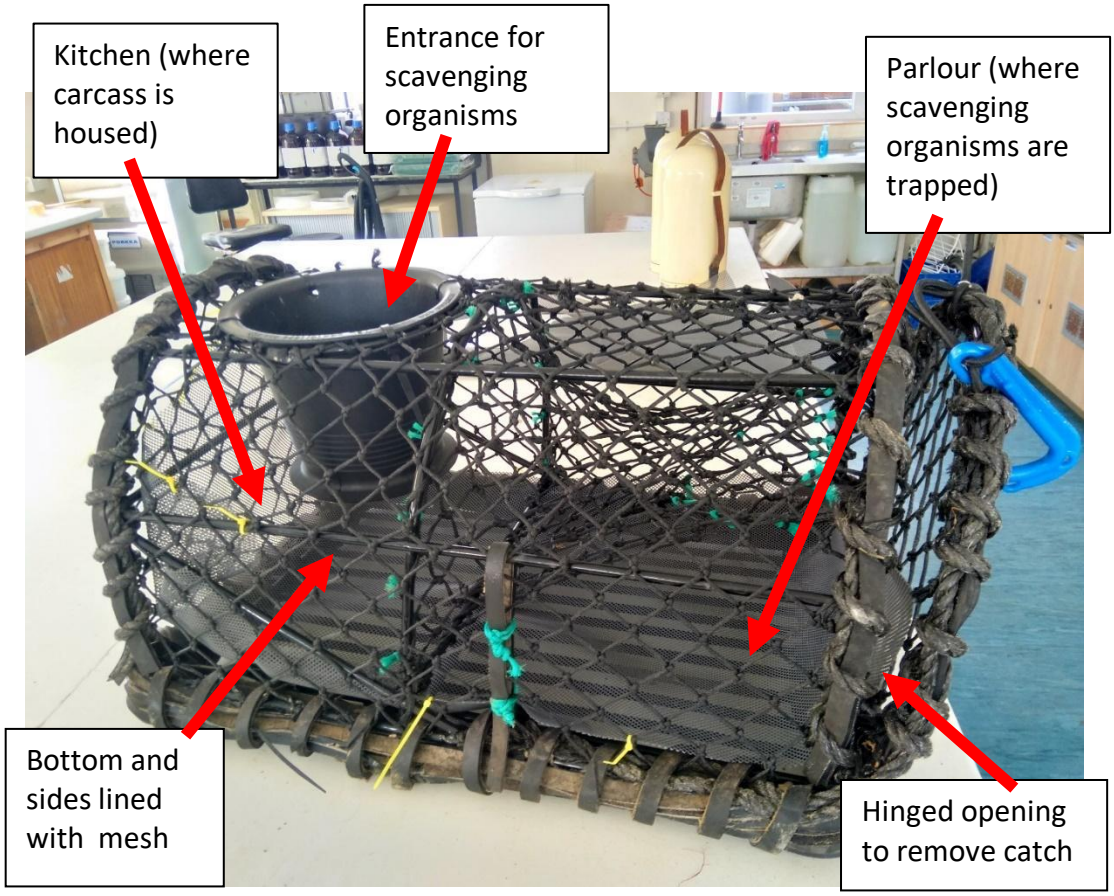

Figure 38: Lobster pot lined on bottom and sides with fine plastic mesh. The hinged narrow end which rested on the sea floor is seen on the right hand side.

5.3.1 Limitations

One particular limitation of the methods discussed here is a lack of replication. Methods 2 and 3 (whelk pot and lobster pot with GoPro™ camera) were only trialled once each. Method 1 was partially replicated twice, with small modifications each time (see section 5.3 above). A full discussion of replication across all studies presented in this thesis can be found in Chapter 8, section 8.3.2 Replication and Pseudoreplication.

5.4 Results

Using all three methods it was possible to record some feeding behaviour as well as the presence of some incidental species. In some instances, crustacean feeding damage was observed even when there were no crustaceans present on the remains when they were removed from the water. This was seen in particular on the lowermost pig in method 1a, as seen in figures 39-42 below, and in the GoPro™ images of the piglet in method 3, shown in figures 43-45.

Figure 39: Feeding damage on lowermost pig in method 1a

Figure 40: Feeding damage on the head and upper back regions of the lowermost pig in method 1a

Figure 41: Feeding damage on the abdominal region of the lowermost pig in method 1a

Figure 42: Feeding damage on the lowermost pig in method 1a

Figure 43: GoPro™ image of feeding damage to piglet in method 3 (first run)

Figure 44: GoPro™ image of feeding damage to body and legs of piglet in method 3 (first run)

Figure 45: GoPro™ image of feeding damage to body and legs of piglet in method 3 (second run)

The modified crayfish pot was unsuccessful at trapping most organisms, although some specimens did remain in the gaps between the plastic mesh of the cage and the carcass. The whelk traps work by attracting whelks inside the pot with bait (in this case the piglet carcass) and once inside the whelks are then unable to climb out. The lobster pot consists of two chambers (the kitchen and the parlour) linked by a one-way entrance leading to scavenging organisms becoming trapped inside the parlour. They can then be released using the hinged opening (Figure 38).

The specimens were identified by Paul Farrell and Graham Malyon (University of Portsmouth Institute of Marine Sciences), or using Sterry and Cleave (2012) and are listed below.

Method 1a (Modified crayfish pot)

- Skeleton shrimp *Caprellidae* sp. Leach (Amphipoda: Corophiidea)
- Scud *Jassa falcata* Montagu (Amphipoda: Ischyroceridae) (Fig. 46)
- Common prawn *Palaemon serratus* Pennant (Decapoda: Palaemonidae)
- Common littoral crab *Carcinus maenas* Linnaeus (Decapoda: Portunidae) (Fig 47)
- Sea squirt Tunicata Lamarck
- Gammarid *Gammarus* sp. Fabricius (Amphipoda: Gammaridae)
- Blenny Wiley & Johnson (Blenniiformes)

Method 1b (Modified crayfish pot with GoPro™ Camera) – *C. maenas*, *Gammarus* sp., *P. serratus*, Blenniiformes sp. (Fig. 48)

Method 2 (Whelk pot) – Common whelk *Buccinum undatum* Linnaeus (Buccinoidea: Buccinidae) (Figs. 49 and 50), velvet crab *Necora puber* Linnaeus (Decapoda: Portunidae) (Fig. 51), northern cowrie *Trivia arctica* Pulteney (Sorbeoconcha: Triviidae) (shell only), common periwinkle *Littorina littorea* Linnaeus (Littorinoidea: Littorinidae), *Gammarus* sp., *C. maenas*

Method 3 (Lobster pot and GoPro™) – *C. maenas, B. undatum*

No patterns were noticed in arrival times of any of the fauna. *Gammarus* sp. was noticed throughout the decomposition period of methods 1 and 1a, but did not appear to be present in methods 2 or 3. This may be due to the design of the equipment as both *Gammarus* sp. and Caprellidae sp. were noted in large numbers collecting on the black plastic mesh of the modified crayfish trap in methods 1 and 1a, as well as on the carcasses themselves. Alternatively, there may be an effect of depth, as methods 2 and 3 involved carcasses at a depth of between 4 and 9 metres depending on tides – however, other species of amphipod have been recorded colonising remains at greater depths (Anderson & Bell, 2017; Dumser & Türkay, 2008; Ellingham et al., 2017).

Figure 46: Jassa falcata collected from uppermost pig (method 1), 7th July 2016

Figure 47: Carcinus maenas collected from pig in whelk trap (method 2) 18th November 2016

Figure 48: Blenny (Blenniiformes sp.) from uppermost pig (Method 1b), 1st February 2017

Figure 49: Common whelks (Buccinum undatum) on piglet in whelk trap (method 2)

Figure 50: Common whelks (Buccinum undatum) collected from piglet in whelk pot (method 2)

Figure 51: Velvet crab (Necora puber) collected from piglet in whelk pot (method 2), 15th December 2016

The cage in methods 1a and 1b was just large enough to house a small carcass without much additional space, and therefore this allowed for unimpeded view for the GoPro™ camera, which resulted in good quality photos showing the carcass being eaten away over time. From these photos it was possible to identify larger organisms that were feeding on the remains. However, in most cases the feeding organisms were not trapped inside the cage as the holes in the mesh were large enough to allow some smaller organisms to easily enter and exist, and the original trap mechanism had been removed thus not allowing larger organisms to enter.

Figure 52: Many of the photographs taken during the winter were poor quality due to the murky water

Many of the photographs taken during the winter field season were poor quality due to the murky water (Fig. 52). However, it is still possible to collect some data and this is important for being able to understand seasonal differences in colonisation. In addition, even if organisms cannot be identified, it is still possible to assess the rate of feeding from the photographs which is helpful for understanding the overall process. Some examples of higher quality GoPro™ images can be seen in figures 53-61. Further images can be found in appendix 9.

Figure 53: Crabs seen on the outside of the lowermost modified crayfish pot, method 1b

Figure 54: A variety of fauna seen on and around the modified crayfish pot used in method 1b

Figure 55: A crab seen on the outside of the modified crayfish pot used in method 1b

Figure 56: Crab scavenging of remains in method 1b

Figure 57: Crabs and whelks seen inside the lobster pot in method 3

Figure 58: A number of crabs seen in the lobster pot in method 3

Figure 59: Crabs seen feeding on the piglet carcass inside the lobster pot in method 3

Figure 60: A large number of crabs seen feeding on the piglet carcass inside the lobster pot in method 3

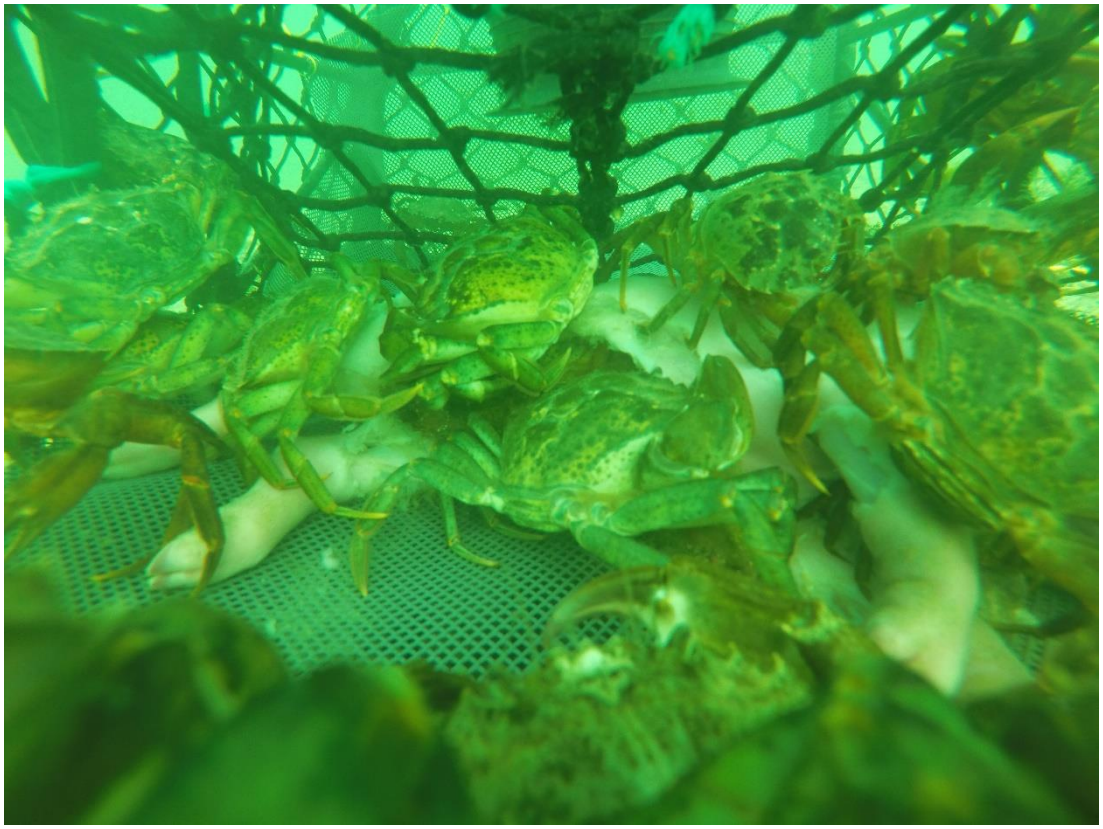

Figure 61: Crabs feeding on the piglet carcass inside the lobster pot, method 3, with two crabs seen scavenging an opening in the abdomen of the carcass

5.5 Discussion

Of the three methods tested, method 3 (lobster pot with GoPro™) was the most successful with method 1b being the next best alternative. The cage in methods 1a and 1b was just large enough to house a small carcass without much additional space, and therefore this allowed for unimpeded view for the GoPro™ camera, which resulted in good quality photos showing the carcass being eaten away over time. This was designed to prevent a larger predator (such as a large lobster) entering the trap and either eating the entire carcass by itself or deterring smaller organisms as this would not happen if the carcass was not enclosed and as such larger predators would be able to come and go rather than remaining with the carcass at all times. Such behaviour has been observed in other related studies, such as Anderson and Bell (2017) in which shortspine thornyhead (*Sebastolobus alascanus*) were observed visiting the carcass periodically to predate on shrimp or occasionally remove pieces of flesh. Despite this some organisms did become trapped inside. In most cases these

were incidental and not seen to be feeding on the remains, however a few *C. maenas* specimens were collected in all three trials and within the first week of decomposition in methods 1b, 2, and 3. This reflects other research where crabs have been reported as a major scavenger, for example in a study by Anderson and Bell (2017) who reported the Dungeness crab (*Metacarcinus magister*) being responsible for removing most of the soft tissue from a carcass during the autumn field season.

Method 3 allowed for more organisms to become trapped, thereby allowing identification of live specimens, however it was more difficult to place the camera to ensure the best quality photos as the space that contained the carcass was much larger which allowed the carcass to move around over time (Fig. 38). There was a range of organisms recorded across the three methods and although there was some crossover with certain organisms appearing in more than one trial, by and large the organisms are different in each case. This demonstrates how different trap designs can affect the types of organisms being collected or recorded.

Amphipods, notably *Gammarus* sp. were recorded across all three methods, and this is in line with previous research and case studies in which feeding damage by amphipods has been recorded. In one study by Vanin & Zancaner (2011) another amphipod, *Niphargus* sp., was recorded and the authors note that both G*ammarus* sp. and *Niphargus* sp. are polyphagous and capable of colonising submerged remains. In the case of bodies recovered after the 2009 Air France 447 crash in the Atlantic Ocean, Ellingham et al. (2017) report that predation occurred by amphipods alongside other organisms such as squat lobsters (*Galatheae*). Dumser & Türkay (2008) also report damage to soft tissue to remains found in the Atlantic Ocean off the coast of Namibia caused by amphipods in the family Lysianassidae but note that in another case in the Mediterranean sea soft tissue removal by amphipods was not present due to a lack of amphipods in the area. Anderson & Bell (2017) also report that Lyssianassidae were immediately attracted to pig carcasses decomposing in the Strait of Georgia, British Colombia, during spring, and were responsible for removing the majority of the soft tissue from the inside of the carcasses. This demonstrates that various amphipod species are likely to be present and cause feeding damage to

remains in areas where amphipods are generally present, and as such investigators should be aware of the species composition in the area of interest.

One limitation of all three methods was that they were all weighted (with the exception of the frame used in method 1a, which was not used in combination with the GoPro™ camera) and therefore remained on the sea floor (approx. 4-9 metres deep depending on tides). This could limit the organisms colonising the remains to benthos, and exclude any pelagic organisms. Therefore, any results obtained by using these methods are only representative of colonisation occurring on remains which are anchored or naturally present at the sea floor as part of the sink/float sequence. However, the design could be adapted to allow the cage to float in the water column by omitting the anchor although this would introduce new variables into the design in the form of varying currents and different ecological regions.

In addition, some of the trials were undertaken in winter during which there were only a few hours of daylight and the water was murky. This meant that there were only a very few photographs in which anything could be seen, and of those many were poor quality due to the murky water (for example Figure 52). Similar results were also noted by Humphreys et al. (2013) who note that automated camera equipment reduces cost but are not suitable for environments without clear water, in which case a diver should be used instead.

The main limitation of this study is the lack of replicates, however the aim was not to provide conclusive data on the decomposition process in the marine environment but to develop a method suitable for use in future small-scale projects. These methods could easily be adapted for larger scale projects simply by using more equipment in order to run multiple experiments concurrently, or by using larger and more professional equipment of the kind seen in Anderson and Bell (2017). Replacing the GoPro™ camera with a more professional still camera may yield better quality photographs, especially in conjunction with lights as this may help to counteract the murky water seen here especially during the winter. In addition, use of larger pig

carcasses would be preferable in order to be more representative of human decomposition.

On occasion aquatic studies have been performed on a very small scale, such as on by Celata (2015) who submerged mice in fresh and marine water inside an incubator, however studies which are more comparable to this one include Chin et al. (2008) in which a piglet carcass was decomposed in a freshwater pond, Humphreys et al. (2013) in which piglet carcasses were placed into crates and submerged in a freshwater reservoir, Pakosh and Rogers (2009) in which dismembered pig limbs were wrapped in plastic bags and submerged in a lake, and Widya, Moffat and Simmons (2012) in which rabbit carcasses were submerged in tap water inside plastic buckets. These studies have primarily been conducted in freshwater, and therefore are not entirely comparable with ones undertaken in a marine environment. As such it was necessary to draw on these methods but also to modify them in order to suit the environment. These studies employ a variety of different methods, as seen in table 5 below. As this type of study is still relatively in its infancy, it is worthwhile testing a number of different methods until they become more established.

Table 5: Comparison of some small-scale aquatic decomposition studies

Reference	Carcass Type	Sample size	Water Type(s)	Submersion Duration	Measurements taken	Notes
Celata (2015)	Mice	54 carcasses	Freshwater and marine water	50 days	Buoyancy of carcasses, weight of carcasses, temperature of carcasses, qualitative measures of decomposition	Mice carcasses chosen to increase sample size; study conducted in incubator
Chin, Marwi, Jeffery, and Omar (2008)	Piglets	1 carcass	Freshwater (manmade pond)	10 days	Climate data – ambient temperature, humidity, water temperature; body surface temperature; maggot mass temperature; entomological samples	Carcass free-floating; carcass was observed until it had completely sunk. Entomological samples taken via sweep netting or manual sampling using forceps
Humphrey, Panacek, Green, and Albers (2013)	Piglets	9 carcasses	Freshwater (reservoir)	2 months	Carcass weight, carcass necropsy (decomposition changes recorded), at-depth water temperatures	Carcass anchored at depth; algal growth was noted on carcasses; no insect activity present
Pakosh and Rogers (2009)	Dismembered pig limbs	120 limbs, two treatments (non-enclosed and plastic enclosed)	Freshwater (lake)	34 days for treatment one and 71 days for treatment two	Weight, length, diameter, and circumference of carcasses; qualitative measures of decomposition; lake temperature and pH	Limbs wrapped in plastic
Widya, Moffat and Simmons (2012)	Rabbits	60 carcasses	Fresh tap water (plastic buckets)	60 days	Presence of adipocere; water and inner body temperatures; soil samples from control (non-submerged) carcasses; water samples from buckets; soil and water pH; decomposition changes using Total Body Score method	Larvae collected from several of the control rabbits; only *Eristalis* sp. noted in buckets

If possible, best results would be obtained through a combination of camera footage and regular checking of traps, however many scavenging crustaceans and other fauna can be identified through photographs taken during daylight hours and the Spring and Summer months. In this case still photography was used, however in other studies film footage has been used instead (for example Anderson & Bell, 2016). These methods could go on to be adopted for larger scale studies by increasing the number of cameras to better monitor feeding organisms. The methods could also easily be applied in other aquatic environments provided the water is deep enough to accommodate the equipment.

Due to the variability in insect colonisation as a result of factors such as temperature and geographical area, there is a clear need for a global approach to forensic entomology. Methods such as those discussed here allow repeated study for essential validation, and therefore help to contribute to the overall understanding of insect colonisation of human remains in aquatic environments.

6. *A Checklist of Arthropods Associated with Piglet Carcasses Decomposing in a Freshwater Pond Environment in Southeast England*

6.1 Statement

Article title: Decomposition and Insect Succession in a Freshwater Pond Environment in Southeast England

Authorship details: Ody, H., Smith, P., and Brown, K.

Publication outlet: Egyptian Journal of Forensic Sciences

Current status: In preparation

The research for this paper was carried out in its entirety by the candidate, with support from the Institute of Criminal Justice Studies technical team. The article has been written in its entirety by the candidate, with support and feedback from Dr Katherine Brown and Dr Paul Smith.

6.2 Abstract

While the process of decomposition in water has been studied, insect succession in different aquatic environments is less well understood. Since insect succession also demonstrates geographic variability, it is important for investigators to fully understand the process in their own location. Insect succession in aquatic environments in the United Kingdom is particularly under-studied, therefore it is important to build a knowledge base on this topic for use in investigations. Here, piglet carcasses were allowed to decompose naturally in a man-made freshwater pond located in a woodland area near Wickham, Hampshire, UK. Throughout the decomposition period, insect specimens were collected and the changes in decomposition state were recorded until the carcasses were fully skeletonised.

6.3 Introduction

Decomposition has been studied in a variety of aquatic environments including ponds and wells (Chin et al. 2008; Dogan et al. 2010; Magni, Borrini, & Dadour, 2013; Sharma & Chandra Bajpai, 2013), canals (Heaton et al., 2010) and marine environments (Anderson & Bell 2014, 2016, 2017; Anderson & Hobischak, 2004; Anderson 2008). However, although it is common to find human remains in water

(Anderson, 2001), much less is known about these processes in aquatic environments compared to on land. Due to this frequency of finding human remains in water, especially in areas with easy access to water (Ahmed et al. 1999), it is important to understand how remains decompose in these environments and be aware of which insect species may be present on remains and therefore be used for PMI_{min} estimation. Research has also shown that there is an operational need for these data in the UK (unpublished data, chapter 3), and specifically for data to be presented in a way that is useful to non-forensic entomologists during investigations. Here, decomposition of piglet (*Sus scrofa domesticus*) carcasses in a manmade freshwater pond was monitored and insect samples were collected throughout. The results are presented in the form of a checklist and annotations are provided discussing key species.

6.4 Materials and Methods

A pond (0.9 x 1.3 x 0.5m) was dug in an area of private deciduous woodland in Shirrell Heath, near Wickham, UK (50°55'04.8"N 1°11'10.2"W). The pond was filled with tap water, and then left to mature for 24 days (24th March – 17th June 2016). A cover made of heavy wooden planks and chicken wire was constructed to cover the pond, in order to prevent access to the remains by scavengers (Fig 62).

Figure 62: A cover made of heavy wooden planks and chicken wire was used to cover the pond

A piglet carcass weighing approximately 500g and measuring approximately 30cm in length (from snout to end of tail) was placed into the pond. The piglet was allowed to decompose naturally and was removed from the pond on 23rd August 2016, 67 days after being placed into the pond. In order to ensure that all the disarticulated remains were collected from the pond, the pond was drained and the debris was sifted for skeletal matter.

For comparison purposes, a dog crate was set up approximately 5m away from the pond (Fig. 63). The base of the dog crate was covered in chicken wire and pegged down with tent pegs. A second piglet (approx. 500g and approx. 37cm in length) was placed inside the crate and the crate was then secured closed using multiple zip ties. The carcass was allowed to decompose naturally, and was fully skeletonised 14 days after placement (17th June 2016 – 1st July 2016).

Figure 63: A dog crate was used to prevent scavenging of the land carcasses

A second set of carcasses was placed out on 10th November 2016, after the pond had had chance to refill with rainwater and settle. These carcasses were also allowed to naturally decompose. The carcass in the dog crate was fully skeletonised on 17th January 2017 (68 days later) while the carcass in the pond was removed on 23rd July 2017 (255 days later).

A third set of carcasses was placed out on 25th July 2017, allowed to decompose naturally, and removed from the pond for the final time on 2nd October 2017 (69 days later).

A fourth and final set was placed out on 26th January 2018. Again the carcasses were allowed to decompose naturally; both sets of remains were removed on 24th May 2018 (119 days later).

Sticky insect traps were placed out with each carcass and replaced each time the carcasses were checked, unless it had failed to trap anything in which case it was left until the next time. Each time, the carcasses were photographed and additional

insect specimens were also taken using a sweep net, manual sampling with forceps, and vial trapping. Air and water temperatures were also taken.

6.5 Results and Discussion

Table 6 contains a complete list of all invertebrate specimens collected from the pond across all four field seasons and using all sampling methods. Table 7 shows a complete list of all specimens collected from the land carcass, again across all four field seasons and using all sampling methods. Specimens were identified morphologically, mostly to family level but to genus and species wherever possible. A number of specimens retrieved from within the pond were highly decomposed and/or fragmented, however any which were too damaged to be identified at all have not been included here. Names shown in red denote specimens which were collected in both environments. Where samples from the same family were collected but have not been identified to genus/species, only the family name has been highlighted.

Table 6: Checklist of all invertebrate specimens collected from the pond environment and pond carcass across all four field seasons and using all sampling methods. Species/Families shown in red were found in both environments. U=unknown.

Class	Sub-class	Order	Sub-order	Superfamily	Family	Subfamily	Genus/species
		Coleoptera			Elateridae		sp.
					Geotrupidae		sp.
					Histeridae	Saprininae	sp.
					Carabidae		*Carabus violaceus*
					Carabidae		*Nebria brevicollis*
					Carabidae		sp.
					Carabidae		*Pterostichus madidus*
					Carabidae		*Platinus assimilis*
					Carabidae		*Pterostichus melanarius*
					Staphylinidae	Aleoch-arinae	sp.
					Staphylinidae		sp.
					Scarabaeidae		sp.
					U		sp.
		Diptera			Syrphidae		sp.
					Syrphidae		sp. (larva)
					Empididae		*Empus lucidus*
					Empididae		sp.
					Muscidae		sp.
					Dryomyzidae		sp.
					Anthomyiidae		sp.
					Phoridae		sp.
					Calliphoridae		*Lucilia* sp.
					Calliphoridae		*Lucilia caesar*
					Calliphoridae		*Lucilia caesar/illustris*
					Calliphoridae		*Lucilia sericata*
					Calliphoridae		*Calliphora vicina*
					Calliphoridae		*Calliphora vomitoria*
					Calliphoridae		sp.
					Calliphoridae		sp. (eggs)
					Calliphoridae		sp. (larva)
					Dolichopopidae		sp.
					Psychodidae		sp.
					Trichoceridae		*Trichocera annulata*
					Scatho-phagidae		sp.

					Heleomyzidae	sp.
					Fanniidae	sp.
					Chironomidae	sp.
					Sarcophagidae	sp.
					Tipulidae	sp.
					Chironomidae	sp. (larva)
					Sphaeroceridae	sp.
					Cecidomyiidae	sp.
					Sarcophagidae	sp.
					U	sp.
		Trichoptera				sp. (larva)
		Hymen-optera			Ichneumonidae	sp.
			Apocrita			sp.
		Orthoptera				sp.
				Aphidoidea		sp.
		Lepidoptera				sp.
		Hemiptera				sp.
		Opiliones				sp.
Arachnida						sp.
	Acari					sp.
Collem-bola						sp.
Chilopoda						sp.
Diplopoda						sp.
Mecoptera						Sp.

Table 7: Checklist of all invertebrate specimens collected from the land environment and land carcass across all four field seasons and using all sampling methods. Species/Families shown in red were found in both environments. U=unknown.

Class	Sub-class	Order	Family	Subfamily	Genus/species
		Diptera	Calliphoridae		*Calliphora vomitoria*
			Calliphoridae		*Lucilia caesar/illustris*
			Calliphoridae		*Lucilia caesar*
			Calliphoridae		*Lucilia* sp
			Calliphoridae		*Lucilia sericata*
			Calliphoridae		*Calliphora vicina*
			Calliphoridae		*Calliphora* sp
			Calliphoridae		sp.
			Calliphoridae		sp. (larva)
			Calliphoridae		sp. (pupa)
			Empididae		sp.
			Muscidae		sp.
			Psychodidae		sp.
			Phoridae		sp.
			Dryomyzidae		sp.
			Hippoboscidae		sp.
			Fanniidae		sp.
			Sepsidae		sp.
			Dolichopopidae		sp.
			Scathophagidae		sp.
			Sarcophagidae		Sp.
		Coleoptera	Elateridae		sp.
			Coccinellidae		sp. (larva)
			U		sp. (larva)
			Silphidae		*Nicrophorus vespilloides*
			Staphylinidae		*Gymnusa brevicollis*
			Staphylinidae		sp.
			Chrysomellidae		sp.
			Histeridae		sp.
			Histeridae	Saprininae	sp.
			Scarabaeidae		sp.
		Hymenoptera			sp.
Arachnida	Acari				sp.

6.5.1 Annotated List of Key Insect Species

Calliphoridae

In both environments Calliphoridae were abundant and among the first species to colonise the remains, as is typical for decomposition studies. The first observed appearances of adult Calliphoridae are listed in table 8 below.

Table 8: First observed instances of adult Calliphoridae in the pond and land environments

		First Observed Appearance of Adult Calliphoridae	
Field Season	**Season**	**Pond Environment**	**Land Environment**
1	Summer	Day 2 – 19.06.16	Day 2 – 19.06.16
2	Winter	Day 1 – 16.11.16	Day 1 – 16.11.16
3	Summer	Day 4 – 30.07.17	Day 1 – 27.07.17
4	Winter	Day 75 – 11.04.18	Day 53 – 20.03.18

In total, the species of Calliphoridae collected were: *Calliphora vicina*, *Calliphora vomitoria, Lucilia sericata, Lucilia caesar,* and *Lucilia caesar/illustris.* All of these species appeared in both environments. Few adult Calliphoridae were collected in the first field season compared to the others, however the most abundant species each field season is presented in table 9.

Table 9: Most abundant species of Calliphoridae in each environment in each field season

		Most abundant species of Calliphoridae	
Field Season	**Season**	**Pond Environment**	**Land Environment**
1	Summer	*Calliphora* sp.*	*Lucilia* sp.
2	Winter	*Calliphora vicina**	*Calliphora vicina*
3	Summer	*Lucilia caesar/illustris*	*Lucilia* sp.
4	Winter	*Calliphora* sp.*	*Calliphora* sp

In this study, *Lucilia sericata*, *Lucilia caesar* and *Lucilia illustris* were all recorded, in comparison with the pilot study (Chapter 4) in which no Luciliinae were recorded at all.

Muscidae

Like Calliphoridae, species of Muscidae were also found in both environments across all four field seasons. This is in keeping with other research and case studies which show Muscid flies appearing on carrion all over the world including Malaysia (Chen et al., 2010; Lee, Krishnasamy, Abdullah, & Jeffery, 2004), Thailand (Sukontason et al., 2007), The United States (Lord, Adkins, & Catts, 1992), Europe (Grzywacz, 2013; Prado e Castro et al., 2012; Velásquez et al., 2012), Turkey (Grzywacz & Pape, 2014), Colombia (Barreto, Burbano, & Barreto, 2002), and Brazil (Barbosa, Mello-Patiu, Mello, & Queiroz, 2009; Carriço, Mendonça, Cortinhas, dos Santos Mallet, & de Carvalho Queiroz, 2015). In these studies, Muscidae are often only identified to genus or family level (Grzywacz, Hall, Pape, & Szpila, 2017).

Carabidae

Several species of Carabidae were collected from the pond environment, usually having fallen into the water and drowned. Although other species of Coleoptera were collected from the land environment, no Carabidae were collected and this was likely due to the choice of sampling methods. Use of pitfall traps in addition to sweep netting, manual sampling, vial trapping, and sticky traps may have yielded greater numbers of Carabidae as the chosen sampling methods are more efficient for flying insects.

Syrphidae

A number of adult and larval specimens of Syrphidae were collected from the pond environment. Although these are not traditionally thought of as being forensically relevant, in more recent years they have been shown to be associated with human remains both in aquatic and terrestrial environments (Archer & Ransom, 2005; Magni, Borrini, et al., 2013; Magni, Pérez-Bañón, et al., 2013; Martins, Neves, Moretti, Godoy, & Thyssen, 2010). Some species are also known to be myiasis-

causing in humans (Aguilera, Cid, Regueiro, Prieto, & Noya, 1999; Salimi, Edalat, Jourabchi, & Oshaghi, 2010; Scott, 1964). Previously, the species of Syrphidae recorded from corpses and grave sites are *Eristalis tenax, Eristalis arbustorum, Brachyopa* sp., *Pseudodorus* sp., *Ornidia obesa*, and *Syritta pipiens* (Lindgren et al., 2015; Magni, Pérez-Bañón, et al., 2013; Martins et al., 2010; Wolff, Uribe, Ortiz, & Duque, 2001). Of these, *Eristalis tenax* and *Syritta pipiens* are widespread in the UK (Nature Spot, n.d.-c, n.d.-b) while *Brachyopa scutellaris* is the most common of the four species of *Brachyopa* present in the UK (Nature Spot, n.d.-a). Three other species, *Brachyopa pilosa, Brachyopa insensilis,* and *Brachyopa bicolor* are more localised and, in the case of *Brachyopa bicolor* and *Brachyopa pilosa*, far more scarce (Ball & Morris, 2013; Nature Spot, n.d.-a; NBN Atlas, 2017a, 2017b, 2017c). Smith (1986) also reports that aphid-feeding larvae of some species in the genus *Syrphus* may also accidentally drop onto remains from vegetation above, and since this study was conducted in a woodland it is not out of the question that this could have occurred. However, these larvae were not observed during this study. Instead, the larvae observed here were 'rat-tailed maggots' (*Eristalis, Eristalinus, Anasimyia, Helophilus, Lejops,* or *Parhelophilus*) (Ball & Morris, 2013). Although larvae of *Eristalis* have been found in association with human remains, their effect on the decomposition process is disputed as they are normally considered to feed on particulate organic matter suspended in the water (Ball & Morris, 2013; Widya et al., 2012).

At the current time, the specimens collected in this study have not been identified, however clearly more attention needs to be paid to the presence of Syrphidae on human remains and the potential for these to be used for PMI or PMSI estimation.

The first observed appearances of Syrphidae larvae and adults are shown in table 10.

Table 10: First observed appearances of Syrphidae adults and larvae

Field Season	Season	First Observed Appearance - Larvae	First Observed Appearance - Adults
1	Summer	Day 1 – 18.06.16	N/A
2	Winter	Day 1 – 16.11.16	Day 147 - 11.04.17
3	Summer	N/A, however pupae observed day 7 – 1.08.17	Day 2 – 27.07.17
4	Winter	Day 41 – 8.3.18	Day 82 - 18.04.18

Trichoceridae

Trichoceridae have previously been recorded on human remains, especially during winter months, and are known to feed on decaying substances in wet environments (Magni, Borrini, et al., 2013; Smith, 1986). Here, adult Trichoceridae were recorded in the pond environment during December of field season 1 and April & May of field season 4.

Psychodidae

Psychodidae are known to breed in wet environments (Smith, 1986) and are commonly found on decomposing remains (Ahmad & Ahmad, 2009; Ahmad et al., 2011; Goff, 2009; Horenstein, Rosso, & García, 2012; Schoenly, Haskell, Hall, & Robert Gbur, 2007; Tantawi, El-Kady, Greenberg, & El-Ghaffar, 1996). In a study comparing several cadavers decomposing in different environments, Lindgren et al. (2015) found that adult Psychodidae were only associated with a cadaver which had been in water and did not appear on other cadavers which were not associated with water. In this study, adult Psychodidae were recovered from both environments although the remains were in relatively close proximity and therefore this may have increased the likelihood of adults visiting both sets of remains. In a study by Tantawi, El-Kady, Greenberg, & El-Ghaffar (1996), Psychodidae were only found on remains during winter. Here, they were found in the pond environment in all four field seasons but in the land environment they were only recorded in the winter, during field seasons 2 and 4.

Dryomyzidae

Dryomyzidae have been recorded on carrion, typically in moist shady areas (Smith, 1986). Here, Dryomyzidae were recorded in both environments, collected via sweep netting or vial trapping.

Coleoptera

While a number of different species of Coleoptera were recorded in both environments (see tables 6 and 7), no aquatic species were recorded in the pond environment. Instead, all specimens collected from the pond were terrestrial and were retrieved from the pond water having fallen in, or from the pond margins. Some species collected are known to be associated with carrion (Silphidae, Staphylinidae, Histeridae, Scarabaeidae, Geotrupidae and Carabidae) (Smith, 1986) while others such as Elateridae, Coccinellidae and Chrysomellidae are incidental and not of forensic importance.

Collembola

One specimen of Collembola was recorded in the pond environment during the first field season. These have been recorded on cadavers found in moist environments (Merritt, Benbow, & Kimbirauskas, 2007; Smith, 1986) but little attention has been given to their potential role in forensic entomology investigations (Merritt et al., 2007).

Mecoptera

Although Mecoptera are not as commonly discussed within forensic entomology literature as some other species, they have nonetheless been recorded colonising human remains (Lindgren et al., 2015), pig carcasses (Albornoz, Ortloff, De, Fuente, & Vivallo, n.d.; Pechal, Benbow, & Tomberlin, 2011), and in bottle traps baited with chicken meat (Rîşnoveanu, Bujor, & Popescu, 2016). Here, two specimens were collected via sweep netting from the pond environment during the third field season (summer, 31st July 2017).

6.5.2 Limitations

This study comprises four replicates per environment across four field seasons. While this yielded a large amount of data in terms of number of insect specimens collected, this is nonetheless a small number of replicates and as such results should be interpreted with caution. While they do provide an overview of which species might be present on or around carcasses decomposing in a small freshwater pond environment, they do not indicate that these species would always be present nor do they attempt to comment on potential presence of successional patterns. They do however provide a baseline of information which can be used for subsequent studies and they represent the first steps towards understanding insect colonisation of remains in small freshwater environments in England.

In addition, for practical reasons it was necessary to decompose each carcass sequentially in the same environment. For this reason, the replication seen here is more accurately described as pseudoreplication (Michaud et al., 2012). This is a problem common to forensic entomology studies and means that particular caution should be applied if applying the results to casework (Michaud et al., 2012).

A full discussion of replication across all studies presented in this thesis can be found in Chapter 8, section 8.3.2 Replication and Pseudoreplication.

6.6 Conclusion

This study represents the first steps towards understanding aquatic decomposition in a stagnant freshwater environment in the South of England. The format for the checklist is based on previous articles, primarily those by Biavati, De Assis Santana, & Pujol-Luz (2010), Carvalho, Thyssen, Linhares, & Palhares (2000), De Jong (1994), De Jong & Chadwick (1997) Goff, Early, Odom, & Tullis (1986), Keshavarzi, Fereidooni, Assareh, & Nasiri (2015), Ramírez-Mora, Buenaventura, Gómez-P, & Amat (2012), Velásquez (2008) and Wolff, Uribe, Ortiz, & Duque (2001). Although this format seems less popular in more recent years, it is still a useful way of presenting data and reflects the amount of work required for knowledge of aquatic forensic entomology to match that of terrestrial forensic entomology. In this case, the data provides a

baseline for future studies into aquatic forensic entomology in freshwater environments in the Portsmouth area.

7. *Effects of Environmental Temperature on Oviposition Behavior in Three Blow Fly Species of Forensic Importance*

7.1 Foreword

The American spelling used in this article is as per the original published version. The abbreviation used here for postmortem inverval is 'mPMI' (also maintained from the original published version), which is equivalent to the abbreviation 'PMI_{min}' used elsewhere in this thesis.

Although submerged remains are colonised by different fauna, floating remains and partially submerged remains are able to be colonised by terrestrial insects including blow flies. Therefore, factors affecting blow fly oviposition, and temperature in particular, are important to the understanding of insect colonisation in aquatic environments. This is particularly applicable to remains found in open water such as larger lakes and the ocean, where temperatures may be lower than those inland.

7.2 Statement

Article title: Effects of environmental temperature on oviposition behavior in three blow fly species of forensic importance

Authorship details: Ody, H., Bulling, M, T., and Barnes, K. M.

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All of the experimental work for this study was carried out by the student as an extension of work completed for the Master of Research (MRes) degree at the University of Derby. The methodology was designed by the student and the PI for the project was Dr Kate Barnes. Statistical analysis was carried out by Dr Mark Bulling (University of Derby) and the paper was written as a collaborative effort by the student, Dr Mark Bulling, and Dr Kate Barnes (University of Derby).

7.3 Abstract

A number of factors are known to affect blow fly behavior with respect to oviposition. Current research indicates that temperature is the most significant factor. However temperature thresholds for oviposition in forensically important blow flies have not been well studied. Here, the oviposition behavior of three species of forensically important blow fly species (*Calliphora vicina*, *Calliphora vomitoria* and *Lucilia sericata,*) was studied under controlled laboratory conditions over a range of temperatures (10 to 40°C). Lower temperature thresholds for oviposition of 16°C and 17.5°C were established for *C. vomitoria* and *L. sericata* respectively, whilst *C. vicina* continued to lay eggs at 10°C. *C. vomitoria* and *L. sericata* both continued to lay eggs at 40°C, whilst the highest temperature at which oviposition occurred in *C. vicina* was 35°C. Within these thresholds there was considerable variation in the number of surviving pupae, with a general pattern of a single peak within the range of temperatures at which eggs were laid, but with the pattern being much less distinct for *L. sericata*.

Keywords

Forensic entomology; Calliphoridae; minimum post-mortem interval; *Calliphora*; *Lucilia*

7.4 Introduction

Developmental stages of key insect species found on corpses can be used to estimate the minimum post-mortem interval (mPMI). As primary colonizers of human remains, blow flies are frequently used in these forensic calculations (Greenberg, 1991; Rodriguez & Bass, 1983). The developmental stages of colonizing insects reached at the time of the discovery of human remains will be the result of two main components: the time between death and the arrival of the colonizers, and the developmental rates of resulting larvae and pupae. Both of these components will vary, depending on habitat and environmental conditions. However, the vast majority of research to date has focused on the effect of environmental factors (mainly temperature) on developmental rates, and determining lower temperature thresholds of development for specific blow fly species (Ames & Turner, 2003;

Anderson, 2000; Byrd & Butler, 1996; Byrd & Allen, 2001; Clark, Evans, & Wall, 2006; Dallwitz, 1984; Davies & Ratcliffe, 1994; Donovan, Hall, Turner, & Moncrieff, 2006; Grassberger & Reiter, 2001, 2002; Higley & Haskell, 2009; Ireland & Turner, 2006; Johl & Anderson, 1996; Kaneshrajah & Turner, 2004; Nabity, Higley, & Heng-Moss, 2007; Wall, French, & Morgan, 1992; Wells & Kurahashi, 1994). Relatively little work has investigated environmental effects on the timing of colonization. Therefore, currently there is a substantial lack in our understanding of how environmental parameters may affect a key component determining the relationship between mPMI estimates and the actual time since death.

The colonization interval (the time between death and colonization) of an exposed corpse can vary from minutes to days for blow flies. Previous research has demonstrated that abiotic factors, including temperature, humidity, solar radiation, rainfall, wind, and light levels can influence blow fly oviposition behaviour (Amendt, Zehner, & Reckel, 2008; Barnes, Grace, & Bulling, 2015; Berg & Benbow, 2013; George, Archer, & Toop, 2013; Mahat, Zafarina, & Jayaprakash, 2009). In particular, temperature has been recognized as one of the most important influences (Amendt et al., 2008; Barnes et al., 2015; George et al., 2013). Temperatures will determine the geographical ranges and densities of blow fly species (Brundage, Bros, & Honda, 2011), as well as influencing their patterns of activity. For example, warmer nocturnal temperatures have been found to encourage blow flies to oviposit at night (Amendt et al., 2008; George, Archer, & Toop, 2013), and Berg & Benbow (2013) found that the abundance of diurnal blow fly oviposition significantly increases when temperatures exceed 20°C on the previous night.

It is generally accepted that colonization by necrophagous flies occurs when air temperatures are between 10°C and 30°C (Amendt et al., 2008; Erzinçlioğlu, 1996; Williams, 1984) although there are exceptions (Erzinclioglu, 1986; Faucherre, Cherix, & Wyss, 1999; Mann, Bass and Meadows, 1990). However, little is known about specific oviposition temperature thresholds for individual blow fly species. In addition, it is likely that oviposition behavior will show regional variation within species, a further complication which could have significant impacts on the utility of

mPMI estimation (Hwang & Turner, 2005) in estimating actual post-mortem interval (PMI).

The time taken for blow fly species to colonise a corpse is likely to be a function of the probability of oviposition which, in turn, will be affected by environmental temperature, solar radiation and the temperature of the corpse. How the probability of oviposition taking place changes with environmental temperature has not been well defined for blow fly species. The local environment at the corpse will also influence the probability of survival of the eggs laid, an aspect which could also be of potential significance for forensic entomology, as differential survival of species will be key in determining the later community structure of colonizers (Pechal, Benbow, Crippen, Tarone, & Tomberlin, 2014). In this study we attempt to define the temperature ranges over which oviposition occurs for three blow fly species of forensic importance. In addition, we determine how the probability of oviposition changes within these ranges, as well as the probability of the survival of eggs laid.

7.5 Materials and Methods

Insect culturing

Colonies of laboratory-bred *Lucilia sericata* (Meigen)*, Calliphora vomitoria* (Robineau-Desvoidy) and *Calliphora vicina* (Linnaeus) were reared in Bugdorm cages (60cm^3) in the insectary at the University of Derby at 20°C ± 3°C, under a 16:8h light: dark photocycle. Each species was bred on a rolling basis from wild-caught parents; the first generation used in the experiment was F1/F2. Flies were fed sugar and water *ad libitum* and provided with porcine liver on emergence and until eggs were laid, providing resources for vitellogenesis and oviposition, following Barnes and Gennard (2013). All adult flies used in these experiments were between 1 and 5 weeks old. Flies for the experiments were only taken from colonies which had demonstrated the ability to oviposit at the time of the experiment.

Oviposition studies

Experiments were conducted from June 2014 to January 2016. Each replicate consisted of 20 adult male flies and 20 adult female flies of the same species being

placed in a meshed cubic cage (length = 30 cm) lined with sawdust. Flies in each cage were provided with 55g ± 0.5g porcine liver, a container of granulated sugar, and a container of water. Cages were placed in an insect growth chamber (IGC) (Fitotron® SGC 120), allowing temperature and humidity to be controlled. Relative humidity was maintained at 55% ± 5% and there was constant light provided by full spectrum light bulbs (4 x 36W fluorescent tubes per shelf, max intensity ~170 µmol m^{-2} s^{-1}).

The first run for each species was conducted at 20˚C (the temperature at which the adult flies had been reared, and the approximate center of the temperature ranges at which oviposition was generally accepted to occur in these three species (Amendt et al., 2008; Erzinçlioğlu, 1996). For each subsequent run, a new cage of flies was used, and the temperature inside the chamber adjusted to the required temperature (sequential 5°C intervals either side of 20°C within the range of 10˚C to 40˚C until the flies would no longer oviposit). Three replicates for each species were run at each temperature (10˚C, 15˚C, 20˚C, 25˚C, 30˚C, 35˚C, and 40˚C). Where oviposition was occurring at one temperature and not at the next, finer increments of 2.5°C and 1°C were then used. In each case the temperature remained constant for 24 hours, after which the presence/absence of eggs was recorded and a new run was undertaken. New adults from the stock populations were used for each replicate.

In addition to recording the presence or absence of eggs after 24 hours, the quantity of eggs (or sometimes larvae at higher temperatures) was estimated. This was done by placing a Petri dish lid with a marked grid (1 cm cells; Figure 64) over the top of the liver and counting the number of squares which contained at least one egg whilst observing from directly above. Although this method does not quantify the number of eggs exactly, it was used as accurately counting the eggs would have required disturbing the eggs. Egg masses are known to generate a structural framework that could influence the distribution of microbes which can potentially alter developmental and survival rates. We therefore avoided complete counting of the eggs as this would have disturbed these processes and potentially affected the key criteria which we were investigating.

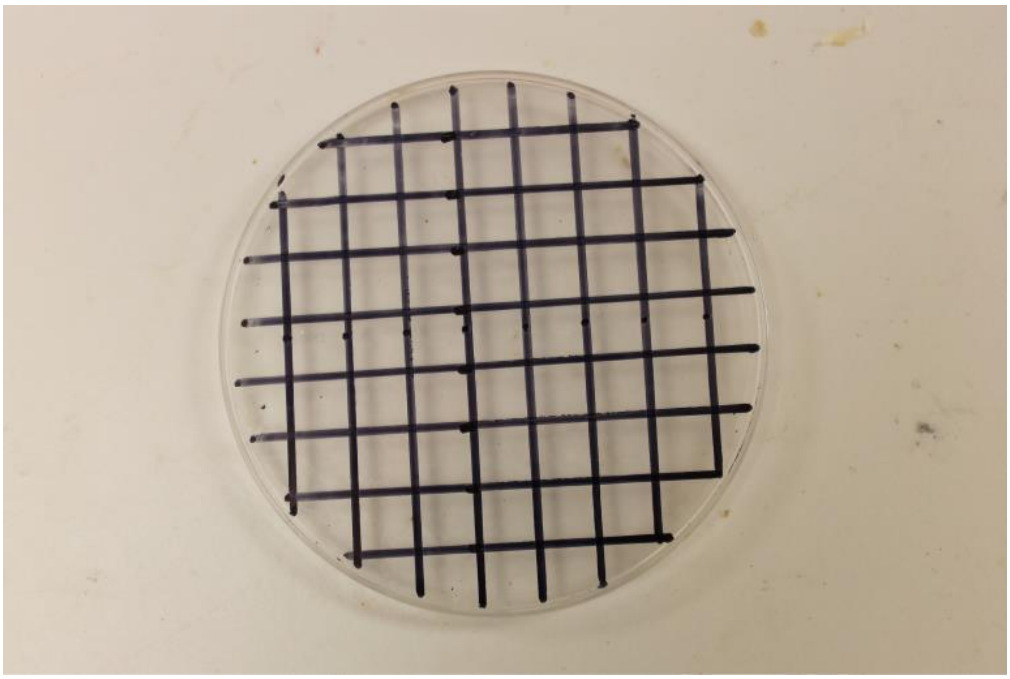

Figure 64: Photograph of the petri dish lid (19cm diameter) with a marked grid of 1cm squares, used to estimate the quantity of eggs laid on the liver

Survival studies

At the end of the 24 hr oviposition period, the liver with the eggs or larvae was carefully transferred onto a thin layer of sawdust (pupation substrate) within clear, plastic tanks and put in a controlled environment (16:8h light: dark cycle, 20 °C ± 3°C temperature). Eggs were reared through to the pupal stage and the resulting number of pupae in each cage was recorded.

Statistical analysis

The presence / absence of eggs for each species was modelled using generalized linear regression models with logit link function and binomial error distributions (logistic regression). The presence / absence of eggs was treated as the dependent variable and the temperature as the independent variable. Given the reasonably complex patterns of the number of squares with at least one egg in, and of the number of surviving pupae, with increasing temperature, both were modelled for each species using a generalized additive model (GAM; (Wood, 2017)). The number of squares/surviving pupae was fitted as a smoother function of temperature. For both the logistic regressions and the GAMs, model assumptions were assessed using residual diagnostics following Wood (2017) and Zuur et al. (Zuur, Ieno, & Smith,

2007). All analyses were conducted in the R statistical programming software (R Core Team, 2016), and the GAMs were developed using the package mgcv (Wood, 2011). Model visualizations were produced using the visreg package (Breheny & Burchett, 2016).

7.6 Results

The lowest temperatures at which oviposition occurred in *C. vomitoria* and *L. sericata* were 16^{o}C and 17.5^{o}C respectively, whilst *C. vicina* continued to lay eggs at 10^{o}C. Both *C. vomitoria* and *L. sericata* were able to lay eggs at 40^{o}C, the highest temperature tested. The highest temperature for oviposition in *C. vicina* was 35°C. Temperature had a significant effect on the probability of egg laying taking place in all three blow fly species (*C. vicina,* LR = 29.42, $p < 0.001$; *C. vomitoria*, LR = 22.49, $p < 0.001$; *L. sericata*, LR = 21.39, $p < 0.001$; Figures 65 and 66). Temperature was also a significant variable in determining the number of cm^2 squares containing at least one egg for all species (*C. vicina,* F = 23.13, $p < 0.001$, explained deviance = 71.1%; *C. vomitoria*, F = 12.88, $p < 0.001$, explained deviance = 64.6%; *L. sericata*, F = 10.35, $p < 0.001$, explained deviance = 67.5%; Figures 65 and 66). However, temperature significantly affected the number of pupae recorded for the *Calliphora* species, but not for *L. sericata* (*C. vicina,* F = 8.52, $p < 0.001$, explained deviance = 64.6%; *C. vomitoria*, F = 4.54, $p < 0.001$, explained deviance = 52.9%; *L. sericata*, F = 1.48, $p = 0.181$, Figure 67).

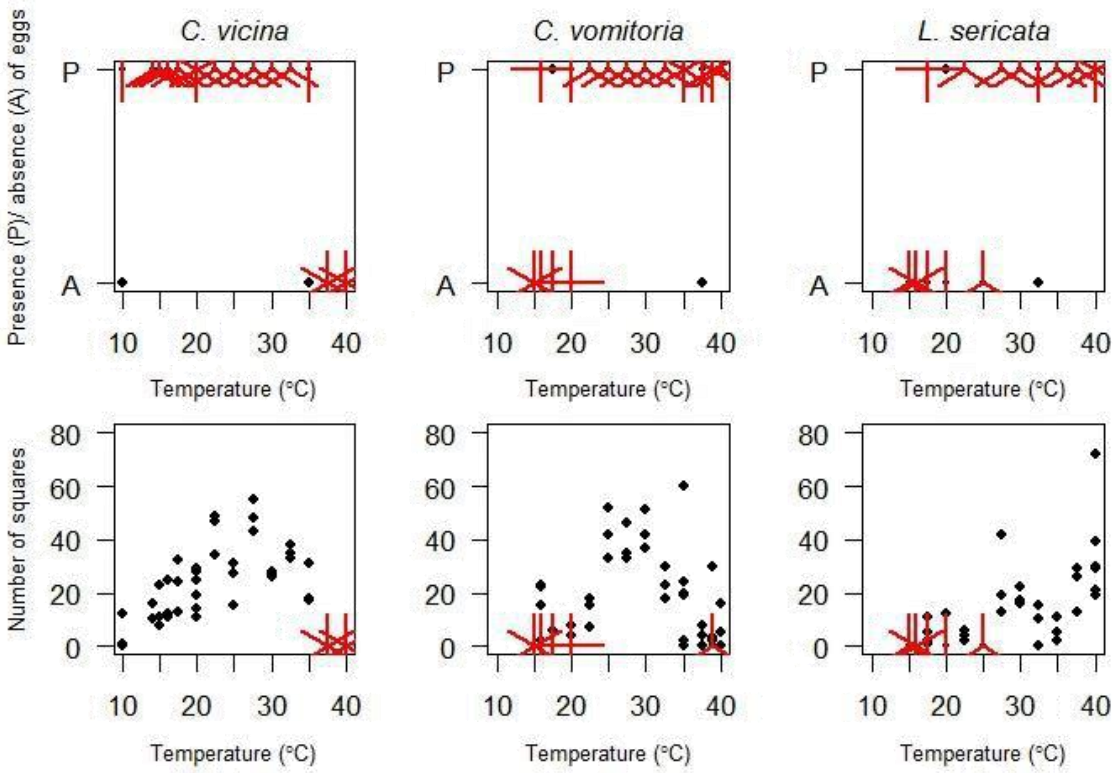

Figure 65: Sunflower plots of the experimental results showing the presence or absence of eggs with increasing temperature (top row), and the number of cm^2 counting squares containing at least one egg at increasing temperatures (bottom row) for each of the blow fly species (columns). The number of arms at each point indicates the number of results. For example, where there are four arms this represents four data points in the same position. A single point represents a single case.

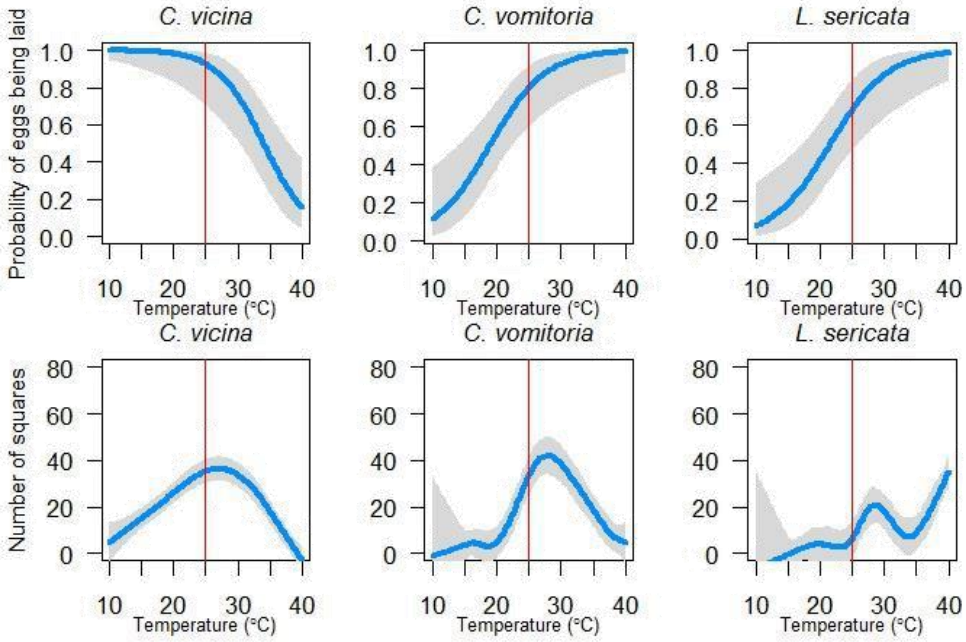

Figure 66: Model visualizations of the logistic models predicting the probability of presence of eggs with increasing temperature (top row), and the generalized additive models predicting the number of cm^2 counting squares containing at least one egg at increasing temperatures (bottom row) for each of the blow fly species (columns). Grey margins indicate 95% confidence intervals. In each plot a vertical (red) line is shown which marks the position of 25°C to act as a guide for comparing model results between species.

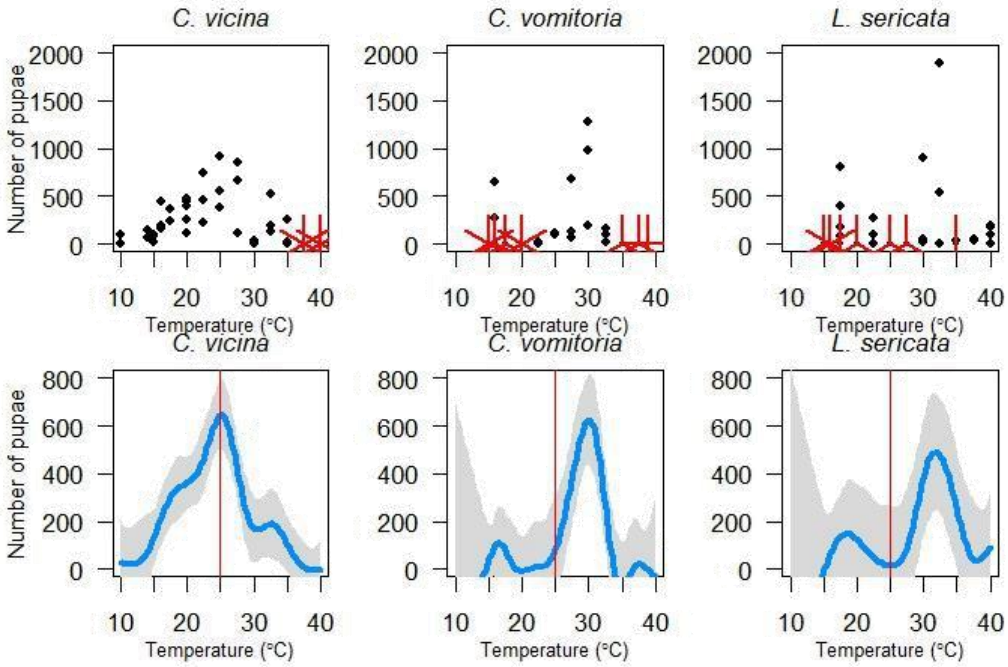

Figure 67: Sunflower plots of the experimental results showing the number of surviving pupae (top row), and the generalised additive models predicting the number of surviving pupae for each of the blow fly species (columns). Grey margins indicate 95% confidence intervals. In each plot a vertical (red) line is shown which marks the position of 25°C, to act as a guide for comparing model results between species. In all plots, the temperature represents the temperature at which the eggs were laid. Following the laying period (24 hr) the egg masses were transferred to an environment of 20°C ± 3oC, under a 16:8 light:dark cycle.

7.7 Discussion

Temperature had a significant effect on the probability of eggs being laid in all three blow fly species in this study. *C. vicina* oviposited at lower temperatures (10 °C, the lowest temperature tested to 35 °C), and *C. vomitoria* (from 16 °C to 40 °C, the highest temperature tested) and *L. sericata* (from 17.5 °C to 40 °C) at higher temperatures. The results for *C. vicina* are consistent with field observations of this species ovipositing at temperatures of 10°C and below (Faucherre et al., 1999). Note that the lower threshold for oviposition for *C. vicina*, and the upper threshold for *L. sericata* and *C. vomitoria*, were not reached within the range of temperatures used in this study.

The greater probabilities of oviposition occurring at the low end of the temperature range used in this study with *C. vicina* compared to the other two species, matches patterns in abundance and activity in other studies. In Germany, *L. sericata* has been associated with corpses over a short, warm period of the year from May to October, *C. vomitoria* from April to November, whereas *C. vicina* was found to be active all year round (Schroeder, Klotzbach, & Püschel, 2003). Hwang & Turner (2005) found activity of *C. vomitoria* and *L. sericata* restricted to the summer months, whilst *C. vicina*, although being most active in summer months, was active from much earlier in the year (April). However, our results contrast with those of Hwang and Turner (2005) when comparing results for *C. vomitoria* and *L. sericata*. Our results suggest that the transition from high to low probability of oviposition occurs at slightly lower temperatures for *C. vomitoria*. However, Hwang & Turner (2005) found *C. vomitoria* activity restricted to mainly June and August, whereas activity for *L. sericata* started later (August) and continued into the colder months (October). We need to be cautious in our interpretation of this difference due to our relatively small data set and the estimated contrast between the oviposition behaviors of the two species in our study being small. However, the habitat preferences of the two species may explain the apparent differences between our results and those of Hwang & Turner (2005). *L. sericata* is associated with urban environments (Brundage et al., 2011; Hwang & Turner, 2005) which will tend to be warmer and therefore potentially maintain preferred temperatures into colder months compared with the rural areas with which *C. vomitoria* is associated (Brundage et al., 2011; Hwang & Turner, 2005). This is supported by Hutchinson (2002) who conducted a study in South East England, and found *C. vicina* and *C. vomitoria* to be active in low numbers during the winter months at an urban study site, but not at their rural pasture site.

We must also recognize that in the wild temperatures will fluctuate, and the extent and consistency of these fluctuations will vary over time, particularly between seasons. However, in our experiment the flies had 24 hours to acclimatize to a constant temperature and oviposit, whereas in the wild they would have several

days, possibly allowing flies to oviposit over greater temperature ranges than found in our study.

The temperature at which the eggs were laid had a significant effect on how many pupae developed for *C. vicina* and *C. vomitoria*, but not for *L. sericata.* The upper temperature threshold for oviposition and puparial survival in *C. vicina* in this study was similar to observations from Donovan et al. (2006) who found that all larvae reared at 35°C died before pupariation. Development studies for several species of Calliphoridae, including *C. vicina*, *C. vomitoria* and *L. sericata* (Anderson, 2000; Greenberg & Tantawi, 1993) tend to be conducted at 20°C or above, which is believed to be favorable or close to optimum for these species (Ames & Turner, 2003). This makes it difficult to compare patterns of oviposition and numbers of pupae in this study with most developmental studies. However, for *L. sericata*, Grassberger and Reiter (2001) reported development rates at temperatures from 17°C to 34°C, indicating that complete development was possible within this range, which coincides with the occurrence of oviposition and patterns of pupae counts in this study. It is important to note that our study differed in methodology compared with these other studies (Ames & Turner, 2003; Anderson, 2000; Donovan et al., 2006; Grassberger & Reiter, 2001; Greenberg & Tantawi, 1993). In our study we varied the temperature at which oviposition occurred, and then reared eggs to pupation at room temperature (20°C ± 3°C), whereas the developmental studies varied the temperatures at which larvae and pupae developed.

Identifying potential causes underlying the distributions of pupae at the different temperatures in this study is hampered by not having exact counts for the number of eggs (due to the requirement of not disturbing them) and due to the period of development, following the initial 24 hr oviposition period, occurring at a consistent single temperature (20 °C). Potentially the number of surviving pupae could be fully explained by the number of eggs laid. The distributions of the number of squares containing at least one egg and the number of pupae match closely for *C. vicina*. However, the range of temperatures over which there are high pupal survival numbers in *C. vomitoria* is considerably narrower than the range over which there were high numbers of squares containing eggs. This strongly suggests that survival

rates decreased rapidly at the edges of the temperature range at which this species showed oviposition behavior. There are two potential reasons for this difference in this study, which cannot be partitioned out. Firstly, the temperature at which the eggs were laid affected the survival rates. Secondly, the temperature change between when the eggs were laid and when they were developing could have affected development and survival. The results for *L. sericata* suggest that the spatial distribution of eggs laid may be important in determining survival rates. At 32.5°C, there were low numbers of squares containing eggs, whereas the largest number of surviving pupae was recorded at this temperature. This suggests that large numbers of eggs were laid in highly aggregated patterns, resulting in greater survival rates. However, as we did not record the exact number of eggs laid, our data are not strong enough to allow us to draw a firm conclusion on this. This may explain why *L. sericata* was the only species for which temperature was not a significant predictor of the number of surviving pupae. However, the spatial distribution of eggs in the experiments was not consistent, and eggs were also laid erratically on other substrates at higher temperatures. This may also have had an effect on survival rates of eggs.

The lowest temperatures at which egg laying took place in the three species tested were higher than the lower development thresholds recorded by other authors. During controlled laboratory studies, Wall et al. (1992) recorded the lower development threshold temperature (DTT) for *L. sericata* as 9°C. In comparison, our study *L. sericata* did not lay eggs below 17.5 °C suggesting that oviposition behavior is restricted to temperatures which are considerably greater than those at which the eggs are capable of developing. *C. vicina* laid eggs over the range of 10 °C to 35 °C. However, the number of squares containing eggs and the number of surviving pupae showed marked peaks centered around 25 °C, with very low numbers at the edges of the range. Similarly, results for *C. vomitoria* showed reduced number of squares containing eggs and number of surviving pupae towards the ranges at which eggs were laid, although there was greater variation towards the lower oviposition temperature threshold.

The developmental stages of colonizing insects reached at the time of the discovery of human remains will be a function of the time between death and the arrival of the colonizers, oviposition behavior, and the developmental rates of the resulting larvae and pupae. Results from this study indicate that temperature will strongly affect oviposition behavior in blow fly species of forensic importance. Importantly, the pattern of oviposition occurring in *C. vicina* was very different compared with the other two species, generally occurring at the lower temperatures. However, our results and comparison with experiments estimating DTTs, suggest that for all three species the range of temperatures over which oviposition occurred was well within the survival tolerances of the eggs. In addition, results indicate that the number of eggs laid, and the survival rates, are likely to vary considerably within the range of temperatures at which oviposition occurs, generally being high over a much more restricted range of temperatures. Previous work (Anderson, 2000; Byrd & Butler, 1996; Dallwitz, 1984; Davies & Ratcliffe, 1994; Donovan et al., 2006; Grassberger & Reiter, 2001, 2002; Johl & Anderson, 1996; Wall et al., 1992) has established that temperature will affect development rates and therefore mPMI estimates. Our work suggests that temperature is also an important determinant of the colonization interval and will lead to variation in colonization times between species. The extent of the colonization interval is important from a forensic perspective as its length will affect the estimate of time of death relative to the mPMI. However, the effects of other factors such as habitat configuration and solar radiation, and their potential interaction effects with temperature on colonization rates, will need to be studied and quantified before colonization intervals by blowflies can be reliably estimated. In addition, the sharp transition between oviposition occurring and not occurring at the upper temperature threshold for *C. vicina* compared with the more variable transitions at the lower thresholds for *C. vomitoria* and *L. sericata*, suggest that data from some species may allow for greater accuracy in mPMI estimates than other data from species over particular temperature ranges.

In this study we have quantified oviposition behavior over a wide range of temperatures for three blow fly species of forensic importance, providing information on how colonizing rates of corpses by these species may be affected by environmental temperature. This provides valuable information for the forensic entomology community, but it is important that the results are viewed within their full context. There is considerable variation in the distribution of blow fly species between habitats, as well as differences between the effects of temperature on blow fly behavior within the same species between geographical regions. This study was conducted using flies collected in the midlands of England, and therefore the use of the thresholds and ranges found should not be extrapolated more widely. However, the broader ecological patterns suggested by this study, such as the nested nature of the distribution of the number of eggs laid and their survivorship, within the range of temperatures at which eggs were laid, are likely to represent more general relationships driven by natural selection. Finally, we have examined only one environmental variable here, whereas other variables such as humidity, wind speed and the presence of rain have been shown to influence oviposition behavior, and offer the potential for interaction effects with temperature. Patterns of the effects of the closely related variables environmental temperature, solar radiation and the temperature of the corpse are likely to be particularly important to determine.

7.8 Conclusion

Temperature significantly affected the probability of oviposition occurring in all three blow fly species, with *C. vicina* having greater probabilities at the lower temperatures and *C. vomitoria* and *L. sericata* having greater probabilities at the higher temperatures. The temperature at which oviposition took place also significantly affected the number of surviving pupae in the *Calliphora* species, but this was not the case for *L. sericata.* These results suggest that temperature will be a significant factor in determining colonization rates of a corpse by blow fly species through its effect on oviposition behavior and will therefore impact on the difference between estimations of mPMIs and the actual time since death. Further work is needed to determine the extent of regional variations in temperature - oviposition relationships

and the nature of potential interaction effects between temperature and other abiotic variables, in order to provide more robust guidance on the extent to which mPMI estimates may vary from the actual time since death.

8. General Discussion

8.1 Summary of Findings

The aim of this thesis was to explore decomposition and insect succession in aquatic environments on piglet (*Sus scrofa domesticus*) cadavers as an analogue for human remains.

The first study presented included a review of relevant literature pertaining to underwater decomposition and faunal colonisation of remains, and demonstrated that despite the presence of useful studies and some relevant findings, overall there is a dearth of research available and there are some geographic locations which are especially poorly provided for. The study also employed the use of a questionnaire and focus group to investigate the current level of knowledge surrounding forensic entomology in aquatic environments, as well as practitioners' opinions on current procedures and level of use of forensic entomology at aquatic death scenes. The results of the questionnaire indicate that there is a relatively low attendance at scenes by entomology experts coupled with a relatively infrequent use of forensic entomology by CSIs, thus leading to potential skill fade and general lack of awareness of techniques, utility, key species etc by professionals such as CSIs and police personnel who are not primarily forensic entomologists. Forensic entomologists who consult on criminal cases report that the quality of specimens they receive for identification (when they have not personally collected it) is sometimes poor and therefore increased current awareness and better communication between professionals and agencies is required.

Four main recommendations were identified using the focus group with SIOs; these being a greater need for prioritisation and proactivity, increased awareness, training & development, increased access of information for police personnel and research which is grounded in the realities of practice. The questionnaire and focus group indicated a clear need for further research to be undertaken with a view towards increased collaboration between agencies and professionals involved in aquatic

death scene investigation, and real-world applicability of results taking into account the pressures and constraints imposed upon investigations.

Overall the results of this study demonstrate the need for further research to be carried out to investigate aquatic decomposition and faunal succession, however it is clear from the results that there is a need for increased communication between forensic entomologists and other forensic practitioners, police officers, and CSIs. Although the police officers who took part in the semi-structured interviews highlighted the quality of communication now compared to in years gone by, they also mentioned that they are not fully aware of the ways in which aquatic forensic entomology could contribute to investigations. In addition, the police officers specifically underscored the need for research to be grounded in investigative practice, to inform the awareness of the wider forensic community and respond to current investigative needs.

From the results of this study it was possible to determine that there is value in undertaking aquatic decomposition and insect succession studies from an operational point of view. As such, the field studies were designed to collect data that could be used during police investigations of underwater death scenes.

Since no other research on this topic has been conducted in the Portsmouth area, it was necessary to start by developing methods which can be easily replicated, and by gathering baseline data which can then be used as a springboard for future research. With this in mind, the first field experiment undertaken was a pilot study in which rabbit carcasses were decomposed in clear plastic lidded boxes containing water from a freshwater stream and from Langstone Harbour (Portsmouth, UK). The results of this study indicated that, in these enclosed environments, the rate of decomposition of the carcasses was comparable across both water types. While the carcass housed in the sea water initially appeared to be decomposing at a faster rate, the one in freshwater sank several weeks before the other. On removing the carcasses at the end of the experimental period it was found that both carcasses were in a similar state of decomposition with comparable amounts of exposed bone

being visible. The sequence of insect succession recorded appeared to be in keeping with previous research, with the ubiquitous UK species *Calliphora vicina* appearing as the dominant species in both environments as well as other common necrophagous species such as *Phaonia subventa* being recorded. In the seawater environment, the time of arrival of these species closely mirrored that recorded in previous studies however in the freshwater environment *Fannia* spp. specimens were collected slightly in advance of Muscidae spp. which are usually the earlier colonisers (Campobasso et al., 2001; Klimesova et al., 2016). However, *Fannia* spp. appeared only one day in advance and therefore this difference is unlikely to be significant and could be put down to an earlier Muscidae presence occurring in between sampling, since sampling was only undertaken once per day. In addition, insect succession is known to vary according to geographical region, local ecology, and amount of sunlight (Amendt et al., 2004; Erzinçlioğlu, 1996; Shean, Messinger, & Papworth, 1993) and therefore a number of abiotic variables could explain this difference. Other species such as Ichneumonidae sp. and Alvsiinae sp. at the expected time based on other research (Grassberger & Frank, 2003).

As this experiment was undertaken in enclosed environments, a great deal of care must clearly be employed in interpreting the study, as the results are unlikely to be applicable to open water or less enclosed aquatic environments. In addition, the use of rabbit carcasses in this study causes the results to be less applicable to human decomposition than other carcass types. As such, it was necessary to proceed with further experimentation in order to investigate decomposition and insect succession in other aquatic environments.

This further experimentation consisted of decomposing piglet carcasses in a harbour (seawater) environment and in a freshwater pond. Ultimately fewer data were collected in the harbour environment compared to the freshwater environment, however several different methods for data collection were tested. The most successful method based on amount of data collected was using a lobster pot to house the carcass along with a GoPro™ camera and Blink Timelapse Controller™ to take still photographs at 30 minute intervals. Due to limitations in the time available

to complete the experiment it was not possible to conduct replications, and therefore the results are limited – however it was possible to demonstrate that this type of research can be conducted using low-budget equipment and therefore a study like this is accessible to smaller labs and students, and therefore there is a greater chance of replication (or at least pseudoreplication) which will provide more robust data. In the freshwater environment, several replications were completed using the same method across different seasons. In this case, a checklist of collected insect species was used to present the information, as the preliminary nature of the experiments (as far as studies conducted in the UK) and close proximity of the carcasses meant that it was not possible to adequately provide successional data at this stage. Some key species are also discussed in more detail, and of particular interest is the presence of Syrphidae adults and larvae. These have not typically been considered forensically relevant, however newer literature has recorded their presence on human remains both on land, and, crucially, in aquatic environments (Magni, Pérez-Bañón, et al., 2013). The data from the freshwater study represent a more robust first step towards understanding decomposition and faunal succession in this environment, however further research is necessary in order to most effectively contribute to police investigations as this is still a comparatively small data set.

As these studies were conducted in the field, environmental factors were not controlled for, although temperature was recorded. Several factors are known to have an effect on insect colonisation of remains, however temperature is one of the environmental conditions which is acknowledged to be the most significant. The last study presented in this thesis comprises a laboratory-based study which investigated temperature thresholds for oviposition in three species of common UK blow fly (*Calliphora vicina, Calliphora vomitoria* and *Lucilia sericata*). While not conducted in water, this study nonetheless provides important information pertaining to blow fly oviposition behaviour at low temperatures, which is relevant in cases where bodies are found in open water as temperatures here are likely to be lower than on land. The results show that the lower temperature thresholds were 16^{o}C and 17.5^{o}c for *Calliphora vomitoria* and *Lucilia sericata* while the lower threshold for *Calliphora*

vicina was 10°C. The highest temperature tested was 40°C, the upper limit of the IGC used for the study, and both *Calliphora vomitoria and Lucilia sericata* were able to oviposit at this temperature. The upper temperature threshold for *Calliphora vicina* was 35°C. As this is a laboratory-based study, the results should be interpreted with caution, however the knowledge can be applied to future studies investigating temperature thresholds for oviposition in the field both in terrestrial and aquatic environments where insects have access to the remains.

These studies represent the first steps towards an understanding of decomposition and insect succession in aquatic environments in the South of England and can be built upon in future experimental work, expanding the scope to investigate specific variables in more detail.

8.2 Practical Applicability to the Wider Criminal Justice Sector

This study was designed with the aim of eventually being able to contribute to police investigations by supplying information about decomposition and faunal succession on remains in different aquatic environments in the South of England. This could be done in the form of providing information about common or 'target' insect or invertebrate species which investigators should be aware of at aquatic death scenes, as well as supplying practical guidance on recovery and packaging of entomological specimens. This has the potential to be provided in the form of either reference material aimed at police officers and CSIs to be used at crime scenes, for example as a quick reference guide such as is already in production for sexual offences, or in the form of an in-person training course. For budgeting and practicality reasons, this could also be packed as a 'module' which could be bolted on to existing training courses, rather than forming a training course in its own right (as budgets for forensic investigation are currently tight (Science and Technology Select Committee, 2019).

As previously highlighted, aquatic death scene investigation is highly multi-disciplinary, and may require the expertise of a wider variety of different professions. Some of these are primarily forensic in nature, while others such as oceanography and marine biology are primarily concerned with other aspects of science but

nonetheless play an important role in this type of investigation (for example in a case described by Ruffell et al., 2017). The research presented here, and other studies like it, serve to demonstrate the complexity of this type of investigation and as such underscore the need for these different agencies and professions to work closely together and to be aware of each other's knowledge and potential to contribute to the investigation.

In addition to investigations which begin with a body being found, knowledge of aquatic decomposition and faunal succession may sometimes be important to other scenarios. In missing persons cases that culminate with the discovery of a body, the practical application is much the same as for investigations where a body is discovered and there is no other information about the circumstances surrounding the death. There may be other evidence available which helps to construct a timeline of the movements of the missing person before their death – for example CCTV or eyewitness reports, but it is still important to understand the events which took place peri-mortem, as well as being able to estimate time since death. As well as informing the police investigation, this may be important to the family of the deceased, although Boss and Carnes (2012) assert that so-called closure, the 'endpoint' of the grieving process, is a myth.

Although the research presented here did not specifically deal with faunal feeding damage (other than to observe it), it is clear from existing research (see section 2.3) that a good understanding of what faunal feeding damage looks like in different environments in order to avoid miscarriages of justice in which faunal feeding damage is confused with another kind of trauma such as human bite marks (Wallace, 2019).

8.2.1 Relevance of Results to Other Types of Investigation

While the field studies were primarily designed to be relevant to death scene investigations involving human remains, the results also have relevance for other types of investigation. Animal forensics is an important and growing area to which methods and knowledge accrued from human forensic entomology studies can be

applied. Ruffell & McKinley (2008) describe a case in which the remains of a diseased calf and several sheep were dumped in a ditch and in a flooded quarry. In this case the sites could not be drained an divers could not be used due to high concentrations of sediment. In this case it was possible to locate the degraded remains of the calf by using GPR antennae mounted in a small rubber boat, further demonstrating the need for investigators to be flexible when faced with recovery of remains from aquatic environments. The intelligence in this case was provided by a farm worker, however when the remains were recovered the calf was significantly more decomposed than anticipated based on the timeline given by the worker. This would be an opportunity to apply knowledge from aquatic forensic entomology and decomposition research to provide a PMI_{min} estimate for the calf. This in turn may help to clarify a series of events and strengthen a case against the perpetrator. It is common to find farm animals such as sheep and cows in water, either following intentional drowning or death due to age or disease (Ruffell et al. 2017). This may be to avoid costs associated with disposal of the carcasses, veterinary costs, or negative publicity for farmers (Ruffell et al., 2017), however other animals are sometimes also dumped in water following death.

Parker et al. (2010) describe another case in which a similar method was used to recover the body of a badger which had been weighed down with rocks and thrown into a ditch. Again this is an example of a situation in which knowledge of aquatic forensic entomology and decomposition could be used to aid an investigation by helping to construct a timeline for the series of events. Occasionally more unusual animals are found in water, with Ruffell et al. (2017) describing one case in which a drug-dealer in Ireland disposed of his deceased pet tiger in a ditch which subsequently filled with water, and another case in which an elephant being removed from Belfast Zoo during the Second World War died and was buried in a creek near Belfast Lough. Some years later when marine erosion led to fears that the elephant might be accidentally uncovered, a search was initiated and the remains were recovered. Although in this case PMI was known for the animal, these cases still serve to illustrate potential other uses for knowledge gathered during aquatic forensic entomology and decomposition studies (Ruffell et al. 2017).

8.3 Costings Involved in Experiments

One of the primary purposes of this research, especially of the study presented in chapter 5, 'A Preliminary Investigation of Faunal Colonisation of Remains in Open Water', is to provide a methodology that it suitable for use in small scale projects, for example student projects and laboratories lacking large budgets. As such, a discussion of the costings involved in each of the field experiments is provided in this section. The costing are based on options available in the United Kingdom and include web links to specific items where necessary.

8.3.1 Influence of Two Enclosed Water Types of Entomological Species Colonisation in Portsmouth, UK

This first experiment is the smallest scale field experiment presented in this thesis and comes with an appropriately small cost. The plastic boxes used to house the carcasses are readily available from homeware retailers and for the particular size used here, retail for around £6-8 each. The rabbit carcasses were purchased from a dog food retailer and are available for approximately £5 per carcass. Similar carcasses are also available for human consumption from butchers at a cost of approximately £8-10 per carcass, however these are prepared for food purposes and are therefore most often sold skinned, in contrast to the furred rabbits used here. In this case the furred rabbits were chosen for their availability and were fit for purpose to assess which insects might be present in the other field studies (and in particular the field study conducted in the pond). The sticky traps used retail at around £3 for a pack of 4 and were cut in half for this study. The rest of the materials used are common laboratory materials (forceps, vials, ethanol, thermometer, sweep net) and as such are likely to be already be available for use.

8.3.2 A Preliminary Investigation of Faunal Colonisation of Remains in Open Water

This study in particular focussed on designing a method that could be used to collect the best possible data at the lowest possible cost. It relies on having access to open water (in this case a harbour) and is likely to also require the use of a boat. This is clearly the main limitation for replicating this study, however it is necessary if the

objective is to study decomposition in open water rather than some other smaller pond or pool. The costs for the other main pieces of equipment used are listed below:

Table 11: Cost of items involved in the harbour field study

Item	Price
Crayfish pot https://store.coastalnets.co.uk/collections/pots/products/crayfish-pots	£228.60 each
Whelk pot https://store.coastalnets.co.uk/collections/pots/products/whelk-pot	£7.80 each
Creel-shaped Lobster Pot https://store.coastalnets.co.uk/collections/pots/products/creel-shape-lobster-pot	£67.80 each
Anchor https://www.fishingmegastore.com/anchors/fisheagle-folding-anchor~7806.html?&utm_medium=shopping&utm_campaign=SHP_GR20&housecode=TA0034&gclid=CjwKCAjwguzzBRBiEiwAgU0FT9vfYkNO3z3knxKcbM3r_fY61BbEVfNPaiHKQbqB6BbzOzAe61NglhoCf4UQAvD_BwE	£25.00 These vary in price by weight and design. Price is for a 3.2kg anchor.
Polypropylene rope https://www.screwfix.com/p/stranded-polypropylene-rope-blue-8mm-x-30m/65017#product_additional_details_container	£8.49 per pack
Chain	Price varies by width and quality. Approx. £8.00/metre
GoPro Hero 4™ Camera https://www.amazon.co.uk/GoPro-CHDHY-401-EU-HERO4-SILVER/dp/B00O1XRT9W	£158.88
GoPro™ Waterproof Housing	£14.99

https://www.amazon.co.uk/Suptig-Replacement-Waterproof-Protective-Underwater/dp/B01G77CQDS/ref=sr_1_6?crid=3MTVH7XSEK5ZX&dchild=1&keywords=gopro+waterproof+case&qid=1587726891&sprefix=gopro+waterproof%2Caps%2C141&sr=8-6	
Blink Time Lapse Controller™ https://timelapse.store/collections/camdo/products/blink-controller-for-hero-3-and-4?gclid=CjwKCAjwnIr1BRAWEiwA6GpwNfq95ppPA4AVUaFvUHfXR2IMlnS3m6h7G_03lhOSY6NWmC3JdaTjQxoCT2gQAvD_BwE	£275.00
GoPro Battery BacPac™ https://www.amazon.co.uk/GoPro-ABPAK-401-Battery-for-Camera/dp/B00NIYJFRO/ref=sr_1_3?dchild=1&keywords=gopro+battery+bacpac&qid=1587727884&s=electronics&sr=1-3	Approx. £40
GoPro BacPac Backdoor™ https://www.amazon.co.uk/SOONSUN-Backdoor-Standard-Housing-Skeleton/dp/B01KXACPF4/ref=sr_1_3?dchild=1&keywords=gopro+backpack+back+door&qid=1587727949&s=electronics&sr=1-3	£10.99

8.3.3 A Checklist of Arthropods Associated with Piglet Carcasses Decomposing in a Freshwater Pond Environment in Southeast England

Although this is a larger scale study, there are few expenses involved. The piglet carcasses used here were provided free of charge by a local pig farmer, however they could be substituted for another carcass type in the event they were unobtainable or prohibitively expensive in another location. The cover for the pond was fashioned from scrap materials, however the components (planks and chicken wire) could be found for relatively low cost at a hardware store. The price of dog crates varies by size and design, however they can be found online for as little as £20. The dog crate is not crucial to the design of this project, and could be replaced with any kind of covering that would allow access by flies and rainfall, but prevent the carcass from being removed by scavengers. The sticky traps used retail at around £3 for a pack of 4 and were cut in half for this study. The rest of the materials used are common laboratory materials as discussed previously.

8.4 Limitations

8.4.1 Carcass Type

One of the main limitations with the study is the type of carcasses chosen for the field research. There is a long history in forensic entomology research of using numerous different animal carcass types including rats (Kočárek, 2003; Szpila et al., 2010; Tomberlin & Adler, 1998; Velásquez, 2008), rabbits (Abouzied, 2014; Bourel et al., 1999; Mahat et al., 2009; Silahuddin, Latif, Kurahashi, Walter, & Heo, 2015; Tantawi et al., 1996), dogs (R. W. Mann, Bass, & Meadows, 1990; O'Flynn, 1983; Reed, 1958), monkeys (Ahmad et al., 2011), piglets (Chin, Marwi, Mohd. Salleh, Jeffery, & Omar, 2007; Payne, 1965), mice (Celata, 2015), chickens (Arnaldos, Romera, Presa, Luna, & Garcia, 2004), and sheep (O'Flynn, 1983) among many others, however the preferred carcass type in lieu of human remains is that of the adult pig. This has remained standard since the 1980s (Matuszewski et al., 2019) because of the similarities between humans and pigs in internal anatomy, fat distribution, diet, and body hair (Schoenly et al., 2006). Although adult pigs are acknowledged as being the best carcass type for this kind of research, it can be difficult to access the resources necessary to be able to use them. For example, they may not be as readily

available as stillborn piglets or carcasses available from butchers or pet food suppliers. It may be difficult to obtain ethical approval to use fully grown pigs, especially if they have been euthanised specifically for research purposes, and there are also regulations imposed by DEFRA for the use, handling, and disposal of animal which vary according to the type and provenance of the remains (DEFRA, 2014). For this research rabbit carcasses sourced from a pet food supplier were used during the pilot study, and stillborn piglet carcasses were used for the freshwater and marine field research studies. Both of these carcass types are more easily accessible than adult pigs and therefore it was possible to conduct repeats of the experiments, which was especially crucial for the main field studies. In addition, amount of space available for research can be more of a problem when using larger carcasses. Experimental sites must be secure and a suitable distance away from other human activity, but there must also be enough space between carcasses to avoid any cross-contamination by crawling larvae (Perez, Haskell, & Wells, 2015). A minimum distance of 100m has been suggested (Schoenly, Griest and Rhine, 1991 cited in Perez et al., 2015) but many studies place their carcasses around 50m apart (including Anderson & VanLaerhoven, 1996a; Archer & Elgar, 2003; Eberhardt & Elliot, 2008; Matuszewski, Bajerlein, Konwerski, & Szpila, 2010; Michaud & Moreau, 2009; Segura, Usaquén, Sánchez, Chuaire, & Bello, 2009; Tabor, Fell, & Brewster, 2005; Voss, Spafford, & Dadour, 2009). Although this seems to be sufficient to avoid cross-contamination by larvae, adult insects are capable of travelling much greater distances and their behaviour may be influenced by having more than one carcass in close proximity, although in reality the impact on this seems to be low (Perez et al., 2015). This is another reason for using the checklist approach to present data from the freshwater pond field study (Chapter 6) rather than presenting succession data, as it was not possible to place carcasses the ideal distance apart for this study. Therefore, to be able to use adult pigs in an optimum setting for forensic entomology research, a research site must be available which is large enough to house fully grown adult pigs placed at least 50m apart from each other, as well as being private land situated a sufficient distance away from other human activity. Clearly there may be difficulties with obtaining such a site, as indeed was the case when selecting a sampling site for the pond-based field study described here. In addition to this, in

order for data from such studies to be applicable to an entire geographic area, the study should be repeated at more than one study site in the region (Michaud, Schoenly, & Moreau, 2012), increasing the difficulty of obtaining access to enough suitable sites.

8.4.2 Replication and Pseudoreplication

Replication

Due to the amount of time required to complete these studies, it was not always possible to include much (or any) replication. Time factors which influenced this were:

a) Amount of time required for carcasses to fully decompose
b) Amount of time required to source equipment and study sites
c) Overall amount of time available to complete the study (i.e. registration time for the Ph.D. award)

The amount of time required for carcasses to fully decompose had an impact primarily on the pilot study (Chapter 4) and the freshwater field study (Chapter 6). In the case of the pilot study it was necessary to collect the preliminary data in order to move on to any of the subsequent field studies, however the carcasses took 151 days (approximately 5 months) to reach an advanced stage of decomposition. At this point the decision was taken to end the study, in order to allow enough time to move on to other components of the research. In the freshwater field study, it was necessary to undertake each replication sequentially (as discussed in section 8.3.2: Pseudoreplication below). The carcasses placed into the pond were allowed to decompose naturally and fully across four field seasons, and took between 67 and 255 days to fully decompose. The carcasses decomposing on land nearby took significantly less time to fully decompose, as is to be expected based on current understanding of decomposition on land and in water, however the decision was taken to wait until the corresponding carcass in the pond had fully decomposed before placing out another carcass on the land. This was to ensure that data collected across both environments were comparable. Due to these lengthy decomposition periods (seen especially in the winter field seasons) only four replicates could be

completed in the available time. However, the amount of time required to source carcasses and a study site for this experiment also played a part. In order to obtain piglet carcasses to use (as an improvement on the rabbit carcasses used for the pilot study) it was necessary to contact several local farms until one was found that was able to supply the piglets. In addition, due to the space required to dig a pond and also place out a dog crate to house the land-based carcass – as well as the need for a private space not accessible to the public – sourcing a suitable study site was also a time-consuming undertaking which delayed the start of the experiment.

In the case of the harbour-based field study, the main limitation was in sourcing the equipment necessary to complete the experiment. In the first instance it was necessary to liaise with the marine biology department within the university to obtain access to the study site, and permission to use their equipment. However, by far the biggest limitation here was availability of a qualified member of staff in order to use the boat and access the study site. Difficulty of access to study sites has been discussed by other authors as a primary reason for a lack of research investigating underwater decomposition and insect succession on carcasses in aquatic environments, and this was experienced first-hand during this experiment.

Despite these limitations resulting in overall less replication than would typically be preferred, the study nonetheless provides preliminary data which can be used as a baseline for future studies. This is especially true now that equipment and study sites have been sourced and can therefore be used again in any subsequent studies without the time delay experienced here.

Pseudoreplication

In both the pond-based and harbour-based field studies, multiple carcasses were used sequentially to collect the data. This method was chosen for practicality reasons as it was not possible to access multiple sampling sites which fit all requirements (private, reasonably easy to access, within reasonable travelling distance from each other). In addition, the University of Portsmouth research raft which was used for the harbour-based studies was only large enough to house one carcass (or set of

carcasses, where metal frames and anchors were used to suspend carcasses at different depths) at a time (figure 28, Chapter 5).

Due to ground coverage by trees and undergrowth, it was also not possible to place multiple spaced out ponds in the private woodland used to conduct the pond-based field study.

Unfortunately, this means that the study does not include any true replication and instead pseudoreplication is occurring (Michaud et al., 2012). This is a problem common to forensic entomology studies and means that the result should be interpreted with caution especially if they were to be extrapolated to casework (Michaud et al., 2012). However, the results still provide value though method development, an insight into insect and invertebrate species present on remains decomposing in aquatic environments in Portsmouth, and creating a basis for other studies in the future which can build and expand upon the data collected here.

8.5 Future Research

Due to the lack of research on this topic compared to other areas of forensic entomology, there are numerous opportunities for future research.

Directly leading on from the research presented here, further repeats of the pond-based field study will allow for a more thorough understanding of insect species likely to colonise remains in this type of environment in the South of England. In addition to this, once enough data has been collected pertaining to likely colonisers, it will then be possible to begin to focus in on other variables in order to build up a full picture which can then be used for estimating PMSI. For example, it may be possible to begin to identify indicator species which are linked to different stages of decomposition. It will also be possible to investigate differences caused by different water depths, temperatures, etc.

Similarly, now that some method development has been completed for the harbour-based study, it will be possible to repeat this study and collect more data as well as

also investigating other factors. As different organisms are likely to be present on remains at different depths, it is worthwhile studying this in more detail. Whale fall studies can be referenced as an additional source of information for remains found at extreme depths which may occur following events such as aviation accidents or diving accidents.

For any future studies it is important to work towards the use of adult pigs as these are a better analogue for human remains than either rabbit carcasses or piglets. However, as previously discussed (section 7.2), it can be difficult to access adult pigs to use in this type of research, particularly in large numbers which would be preferred in order to study multiple carcasses concurrently as well as being able to perform repeats. There are also issues with space, as clearly adult pigs take up more space than smaller carcasses. In this case, the private woodland used to conduct the pond-based study has dense coverage of trees and undergrowth meaning that it would be difficult to find enough space to house a large enough pond to fit a fully-grown adult pig without clearing a larger area of the woodland. For this reason, it would be necessary to locate a new research site with enough space but also no access to members of the public.

Survival of blowfly larvae and pupae in water has been studied (Reigada, Gião, Galindo, & Godoy, 2011; Singh & Bala, 2014; Singh & Greenberg, 1994). While undertaking the pilot study it was observed that blow fly eggs laid on the surface of a floating carcass might be partially or temporarily submerged with water lapping over the remains. As this situation might be common where remains are only partially submerged, floating at the surface, or secured at the surface in some way, it is important to know whether eggs which have been wet for a given period of time may subsequently hatch. According to Hinton (1969), insect eggs which become submerged due to rain for hours or even days at a time are likely to be adapted for respiration in water. One important factor to note is that this water is usually aerated, allowing the eggs to respire using a gill called a plastron (Hinton, 1969) and in fact *Calliphora* eggs will die in de-aerated water (Anderson, 1960). In response to this information and the observations made during the pilot study, an experiment

was set up to investigate survival of blow fly eggs submerged in different amounts of water and for different lengths of time (see appendix). Although this study is very much in its infancy, this is an important avenue to revisit based on observations made during the field studies contained in this thesis.

As suggested by the semi-structured interviews, it is important that the results of this research and any subsequent research are communicated not only to the scientific community but also to CSIs and other police personnel. It is important that this information is presented in a format that is easy to understand for non-specialists, as well as easy to use during investigations. Specialist training sessions in aquatic forensic entomology could be provided to 'top-up' knowledge for CSIs to ensure that current knowledge is being used in practice.

8.6 Conclusion

This study has made the first steps towards understanding decomposition and insect succession in aquatic environments in the South of England. Chapter 3, Forensic Entomology and Underwater Death Investigation: A Review of its Utilisation and Potential demonstrates that there is a need for future research into aquatic decomposition and faunal colonisation, and suggests that police personnel in general are receptive to and understand the importance of engaging with researchers. This study also highlights the overall lack of research on this topic and as such underscores the need for a greater understanding and for more research to be produced, which should be designed with investigative needs in mind.

While there is more work to be done in order to build a full picture, it has been possible to collect some data pertaining to insect and invertebrate species likely to colonise remains decomposing in fresh or sea water in the area. This was achieved through the three field studies and provides a base to which further data can be added in the future.

Overall it is possible to make several recommendations from the studies presented in this thesis. As previously discussed, the study in chapter 3 has emphasised the

need for more research to take place and for this research to be applicable to investigations. The field studies demonstrate that, while it is important for methods to be robust and standardised, researchers and investigators may also need to take a flexible approach to working in order to cope with the complexities of aquatic environments. However, the studies have also demonstrated that it is possible to carry out this type of research on a small budget but again that a level of flexibility is needed to compensate for a lack of expensive and highly specialised equipment.

There is scope for future research using the same methods to collect additional data, as well as exploring beyond faunal colonisation, for example to investigate survival of the eggs of common UK blow fly species after submergence in water.

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University of Portsmouth

Faculty of Humanities and Social Sciences

Application for Ethical Review – Staff and Postgraduate Research Students

1. Study Title and Key Dates

1.1 Title: The study and application of underwater decomposition from an entomological perspective for the purpose of post-mortem interval estimation
1.2 Date of submission: **Version Number: 1** **Ethics Committee Reference Number:** 15/16: 29 **1.3 Date of study commencement: 30th September 2015** **Projected date of study completion (fully written up): 30th September 2018**

2. Applicant Details: Please complete either 2.1 or 2.2 as appropriate

2.1 Principal Investigator (Member of staff –personally or as a supervisor of a taught student)
Name: Helen Ody **Title /Role: PhD Student** **Department: ICJS** **Telephone: 02392 845058** **Email: helen.ody@port.ac.uk**
2.2 Principal Investigator (PGRS)
Name: Helen Ody **Title /Role: PhD Student** **Department: ICJS** **Course of study: PhD Forensic Entomology** **Telephone: 02392 845058** **Email: helen.ody@port.ac.uk** **First Supervisor's Name:** Dr. Katherine Brown **Telephone:** 02392845247 **Email:** katherine.brown@port.ac.uk **Names and contact details of any other supervisors (if relevant)** Dr. Paul Smith; paul1.smith@port.ac.uk (second supervisor) Dr Francis Pakes; francis.pakes@port.ac.uk
2.3 Co-Researchers / Collaborators
N/A – this project is being entirely undertaken by the student and supervised by Dr. Katherine Brown and Dr. Paul Smith.
2.4 Independent or Peer Reviewer
Role fulfilled by academic supervisors (see above).

4. Funding Details

Fully funded by the University of Portsmouth.

5. Research Sites

University of Portsmouth Institute of Marine Sciences – raft in Langstone Harbour – for sea water studies and possibly freshwater studies (depending on availability of equipment) Woodland near Wickham – for terrestrial and freshwater studies. Grounds of Ravelin House, University of Portsmouth – for terrestrial, freshwater, and saltwater pilot studies Risk assessment forms available in appendix.

6. Insurance Arrangements

N/A – normal university insurance is sufficient.

7. Study Summary

6.1 Study Summary
The study is designed to provide location-specific data on invertebrate succession (the sequence in which insects colonise cadavers and the resultant changes to the communities of insects present on the remains over time [Lopes de Carvalho and Linhares 2001]) and decompositional changes to remains found on land, in freshwater, and in salt (sea) water. The data collected during this study will be used to narrow down minimum post-mortem interval (mPMI [=time since death]) estimations calculated by entomological and taphonomical means for the Portsmouth area. mPMI determination using insect evidence is an established technique within forensic science, however since insect behaviour varies according to geographic location (Campobasso *et al.* 2001, Amendt *et al.* 2004), it is recommended that the forensic entomologist collects their own data for the area in which they are working. Studying insect succession on cadavers in Portsmouth, UK, will therefore provide information relating to the specific sequence of insect succession as well as the species composition of local forensically important arthropods in the area. In addition, insect succession in underwater environments has not been studied in great detail (Amendt *et al.* 2004), therefore this research will also provide additional data on this topic. It is common for human remains to be found in water, with deaths resulting from accidents during recreation and disposal of remains following murders (Anderson 2002. Suicide by drowning also occurs, especially in areas with easy access to water (Wirthwein 2002). Therefore, it is necessary for forensic entomologists based in the South coast of England to have an understanding of both terrestrial and aquatic faunal succession in the area. While insect succession varies according to geographical region, the results of this study have the potential to be applied to other temperate coastal regions or to provide a starting point for researchers wishing to investigate insect succession on remains in terrestrial and aquatic habitats in other geographical zones. While faunal succession in water is known to be very different to that on land (Anderson 2002), few studies have been conducted examining development, species composition, and successional patterns of invertebrates in aquatic environments. Hobischak (1997) and MacDonell and Anderson (1997) found differences in faunal succession between different types of habitat, as well as differences caused by other factors. Chin *et al.* (2008) note that insect species recorded in their study conducted on a decomposing piglet (*Sus scrofa*) in a manmade freshwater pond differed from species recorded by Hobischak (1997) and others (Vance *et al.* 1995; Keiper *et al.* 1997), in other parts of the world. This supports the necessity of investigating and comparing faunal succession in terrestrial and aquatic habitats in the South of England. For this study, at least one piglet (*Sus scrofa*) carcass will be sampled per habitat (as per Lopes de Carvalho and Linhares 2001, Hobischak and Anderson 2002, Anderson and Hobischak 2003, Matuszewski *et al.* 2008, and Barrios and Wolff 2011, for example). These carcasses are then sampled over a period of at least one month (Keiper *et al.* 1997, Matuszewski *et al.* 2008) or until skeletonisation occurs (up to one year) (Lopes de Carvalho and Linhares 2001, Hobischak and Anderson 2002, Anderson and Hobischak 2003, Barrios and Wolff 2011). On each sampling occasion, the carcass will be photographed and the state of the carcass documented (Hobischak and Anderson 2002, Matuszewski *et al.* 2008, Barrios and Wolff 2011) and insect sampling can be undertaken. Other data including water samples, climatological data, and soil samples will also be collected in order to monitor changes in the soil and water microorganisms throughout the decomposition process (Lopes de Carvalho and Linhares 2001, Hobischak and Anderson 2002, Chin *et al.* 2008, Voss *et al.* 2009, Barrios and Wolff 2011). Immature insects taken during

the sampling process will be reared and identified in the laboratory. Following skeletonisation, the remains will be left in their respective environments and monitored for insect damage to see whether certain species of beetle have a role in the decomposition process after remains are skeletonised (Holden *et al.* 2013).

In addition to this, questionnaires and interviews will be used to explore current approaches to recovery of human remains from water, including the application of forensic entomology (how many cases employ the use of entomology, whether entomology is being under-used, the outcomes of using forensic entomology). The data from these questionnaires and interviews will be analysed alongside statistics from the Office of National Statistics pertaining to the number of registered deaths by accidental drowning or submersion. These statistics are freely available in the public domain for the years 2008-2014.

6.2 Main Ethical Issues

1. Sensitive/personal data - the questionnaire and interview portion of the study is designed to collect information regarding current procedures and outcomes of cases involving recovery of remains (or rescues of live persons) from water; this information specifically pertains to the procedures for recovery and the use of forensic entomology in these cases. The questionnaires and surveys will help to provide context for the data collected through field research. Neither the questionnaire nor the interviews are designed to collect sensitive data, however all data will be treated carefully and appropriately. This will include anonymising data where appropriate, not publishing any details which could identify participants, and taking care to store data in a safe and secure manner in a locked filing cabinet (paper copies) and a password protected removable hard drive.
2. Risk of disclosing unprosecuted crimes – as stated above, the questionnaire and interview are not designed to collect any personal or identifying data about any specific crimes, unprosecuted or otherwise. All data will be treated in a sensitive and appropriate manner and no details will be published which could disclose or identify any specific crime.
3. Use of animal remains – All piglets used during the study will be “waste” piglets, i.e. stillborn or those which have died by being crushed by the sow. The remains will be treated respectfully, kept in the freezer until required, and disposed of in a safe, respectful, and humane manner. This will involve allowing the remains to decompose naturally to the point of skeletonisation, and following the monitoring of the skeletal remains for insect activity, the bones will be disposed of in landfill which is acceptable according to guidelines laid out by Trading Standards (Hampshire County Council, ND).

6.3 Other Risks or Concerns

The research does not risk damage to the university’s reputation, or involve any conflicts of interest.

The piglets in the harbour will be fully submerged apart from the ones at the shallowest depth which may float to the surface as gases are released during the decomposition process. All the piglets will be underneath the raft owned by the IMS. The raft is only accessible by boat and should therefore not be easily accessible by members of the public. In addition, the containers which will be used to house the piglet carcasses are made of a thick black plastic mesh which should help to conceal the contents. These may also be wrapped in black bin liner plastic (to be decided upon discussion with/advice from staff at the marine biology centre) which would conceal the contents completely. The woodland which will house the freshwater and terrestrial experiments is private and is therefore not accessible at all to members of the public.

8. Compliance With Codes, Guidance, Policies and Procedures

This research will fully comply with the Concordat to Support Research Integrity, RCUK Policy, and Guidelines on Governance of Good Research Conduct. There are no other policies and procedures for conducting this research as the study is based on that of other researchers to maximise data gathering with minimal use of animals.

9. Study Aims and Objectives

8.1 Main Aim / Research Question/Hypothesis
To investigate invertebrate succession on remains found on land, in freshwater, and in sea water for use in forensic death scene investigations.
8.2 Primary Objective
To identify the sequence of invertebrate succession on pig (*Sus scrofa*) cadavers on land, in freshwater, and in sea water in order to provide location-specific data and help narrow down mPMI (minimum post-mortem interval, time since death) estimations for the South Coast area.
8.3 Secondary Objective(s)
1. To place the research into context with other similar research and with forensic practice by conducting interviews and questionnaires with practitioners and others in relevant occupations. 2. To identify changes in the soil and water microbiome throughout the decompositional period. 3. To monitor bones for invertebrate damage following skeletonisation. 4. To inform investigative strategy and police procedure on estimation of mPMI of cadavers in water

10. Research Methods

9.1 Research Method(s)
Survey – to be conducted electronically via Surveymonkey and on paper with participants mailing or emailing copies back. Draft appended. Interviews – interviews will last for a maximum of one hour dependent on length of respondant answers to questions. Interviews will consist of approximately 6 questions. The interviews are not designed to collect any sensitive data, however some may be given in the open questions. Draft appended. Observation, sampling, and laboratory work – the piglet carcasses will be observed throughout the decomposition process and samples of insects and water/soil will be collected. Weather data (temperatures, wind speed, rainfall etc) will be collected using thermometers, data loggers, and publically available data from local weather stations. Sampling will be conducted daily where possible initially (reducing with time from carcass placement), and otherwise as often as possible dependent on availability of the boat belonging to the Institute of Marine Sciences.

A pilot for the study will be undertaken, consisting of observation and sampling as described above of rabbit carcasses placed in plastic boxes and situated in Ravelin gardens.

Prior to designing the questionnaire and interviews, a forensic practitioner and spokesperson for the RNLI were contacted to assess their willingness to potentially be involved. Both parties indicated that they would be willing to consider taking part in the research once ethical approval has been sought.

10. Recruitment of Participants

10.1 General Considerations
Potential participants will be identified by their occupation – i.e. practicing forensic entomologists involved with the European Association for Forensic Entomology, volunteer with the RNLI, member of relevant emergency services (fire service, police). Participation will be entirely voluntary.
10.2 The Research Population
The research population will consist of members of EAFE, RNLI volunteers, fire officers, police officers. None of the members of the research population have been involved in the research design, nor have they been invited to review participant documents. The research population will be approximately 200 people.
10.3 Sampling Strategy
Inclusion criteria (purposive sampling): participants will all be practicing forensic entomologists (members of European Association for Forensic Entomology), volunteers with RNLI, or members of the fire service or police service. Sample size: minimum response rate of 5 people per profession Participant involvement will be for the length of the questionnaire and interview.

10.4 Recruitment Strategy – Invitations to Potential Participants
Participants will be recruited through sending letters of invitation (appended) to people in relevant professions. For members of EAFE, letters of invitation will be distributed using a list of members (available through their website; if an updated list is required, the chair of the society can act as a gatekeeper to distribute invitation letters to current members). Invitation letters will be distributed to members of the police force via the University of Portsmouth's links with Hampshire Constabulary and via members of UOP ICJS teaching staff who have contacts within other constabularies and who will be able to suggest potential gatekeepers. Members of the RNLI and fire service will be contacted through their marketing/PR departments where the appropriate member of staff will act as the gatekeeper and pass the paperwork onto employees. Payment will not be offered to any participants in order to maintain anonymity and confidentiality.

10.5 Obtaining Consent
In order to gain consent, participants undertaking the questionnaire on paper will be provided with a participant information sheet and two consent forms – one relating to the questionnaire and one relating to the interview. The participant information sheet will supply participants with the details of the study (an overview of the study and their potential involvement) and the consent form will cover their participation in the questionnaires and verbal interviews. Participants undertaking the questionnaire online will be provided with an electronic copy of the participant information sheet and the two consent forms (integrated into the online version of the survey). No respondent details are required other than the participant's job role. Paper copies of the information sheets,

consent forms, and questionnaires will be numbered to ensure that the correct paperwork has been filled out for each participant (i.e. consent form 001 corresponds to questionnaire 001 etc.)

Completed consent forms will be stored in accordance with guidelines, i.e. electronic copies will be held on the university N drive, a specific password protected external hard drive and hard copies in a locked filing cabinet.

Documents appended.

10.6 Organisational Consent
N/A – no research will be conducted in any external organisation.
10.7 Participant Withdrawal
Participants will be given the option to withdraw at any stage of the research prior to data analysis (on request of participant; no later than December 2017).

11. Research Data Management

11.1 General
The data steward for this research will be Professor Francis Pakes. Costs involved with the project will be minimal but external funding will be sought wherever necessary/practical. Data generated during the study will be stored securely on the university N drive and archived for 10 years as per university policy; it will also be made open-access and available in the university research data repository and licensed for reuse and sharing if published.
11.2 Data Collection and Analysis
Data collected will be: • Survey data • Interview recordings and transcripts • Demographic data (occupation only) • Data from field experiments and lab work (species composition data, weather data, decomposition stages) Data will be analysed using a mixed-methods approach, including thematic and content analysis, and descriptive and inferential statistics. Laboratory and field research will involve microscopy, microbiology techniques such as agar plating for bacteria, and entomological collection techniques.

11.3 Data Storage
I will store data as per the Data Protection Act 1998 and the University of Portsmouth Research Data Management Policy.
11.4 Destruction, Retention and Reuse of Data
Retention, destruction, and reuse of data will be as per the Data Retention Act 1998. Although the study is not designed to specifically collect any personal data, all personal data associated with the study (e.g names of participants to the interviews) will be destroyed following the study and any published data will be retained for 10 years and stored on the university N drive.

11.5 Personal Data – Confidentiality and Anonymisation
N/A – it is not the intention to collect or use any data which might identify participants beyond their occupation. Should any data which could potentially identify participants be collected during the questionnaire/interview section of the study, this data will not be used in such a way as could identify any participants. Additionally, it is not the intention to identify any participants via IP or email addresses, although this could theoretically be possible (for example if participants choose to return their completed questionnaires via email). When conducting interviews with participants, it will not be possible to collect this data anonymously (due to needing to contact participants prior to interviewing and speak with participants during the interviews) however none of the data collected will be used in such a way as would identify participants, again beyond their occupation. Any data collected (whether personal or not) will be stored securely on the University N drive (and as described above). Access to these data will only be possible for myself and may also be viewed by the project supervisors.

11.6 Organisational Data
N/A – any organisational data used will either be from the public domain and appropriately referenced, or will be obtained via questionnaires/interviews which will be stored securely as previously described.

11.7 Security Sensitive Data
N/A – no access to security sensitive documents is required.

12. Risks

12.1 Risks to Participants
N/A – participants are not required to undertake any activity which might carry any physical or emotional risks.
12.2 Risks to Researchers
• Lone working • Travelling by boat/working on the raft • Handling insects • Use of a sharp knife for cutting meat during rearing of insects Risk assessment appended.

13. Publication Plans

It is the intention to publish the results of this research as several papers in scientific journals. The results of this research are intended to influence police procedure when investigating death scenes in aquatic environments by informing police personnel about which invertebrate species to collect

as evidence at the scene, and providing location-specific insect succession data to help narrow mPMI estimates based on insect evidence.

14. References

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15. Appendices

Study Title: The study and application of underwater decomposition from an entomological perspective for the purpose of post-mortem interval estimation

Document	**Date**	**Version No.**
Application Form		
Participant Information Sheet(s) (list if necessary)		
Consent Form(s) (list if necessary)		
Invitation Letter		
Advertisement	N/A	
Peer / Independent Review	N/A	
Research Data Management Plan		
Supervisor Email Confirming Application		
Evidence From External Organisation Showing Support	N/A	
Terms of Reference to Steering / Advisory Group	N/A	
Survey Instrument		
Interview Questions / Topic List		
Focus Group Questions / Topic List	N/A	
Focus Group Ground Rules	N/A	
Script for Oral Consent	N/A	

Questionnaire		
Observational Data Collection Form	N/A	
Risk Assessment Form		
Principal Investigator's Response to the Ethics Committee		
Other – please describe		

16. Declaration

Declaration by Principal Investigator, and, if necessary, the Supervisor

1. The information in this form is accurate to the best of my/our knowledge and belief and I/we take full responsibility for it.
2. I/we undertake to conduct the research in compliance with the University of Portsmouth Ethics Policy, UUK Concordat to Support Research Integrity, the UKRIO Code of Practice and any other guidance I/we have referred to in this application.
3. If the research is given a favourable opinion I/we undertake to adhere to the study protocol, the terms of the full application as approved and any conditions set out by the Ethics Committee in giving its favourable opinion.
4. I/we undertake to notify the Ethics Committee of substantial amendments to the protocol or the terms of the approved application, and to seek a favourable opinion before implementing the amendment.
5. I/we undertake to submit annual progress reports (if the study is of more than a year's duration) setting out the progress of the research, as required by the Ethics Committee.
6. I/we undertake to inform the Ethics Committee when the study is complete and provide a declaration accordingly.
7. I/we am/are aware of my/our responsibility to be up to date and comply with the requirements of the law and relevant guidelines relating to security and confidentiality of personal data, including the need to register, when necessary, with the appropriate Data Protection Officer. I/we understand that I/we am/are not permitted to disclose identifiable data to third parties unless the disclosure has the consent of the data subject.
8. I/we undertake to comply with the University of Portsmouth Research Data Management Policy.
9. I /we understand that research records/data may be subject to inspection by internal and external bodies for audit purposes if required.
10. I/we understand that any personal data in this application will be held by the Ethics Committee, its Administrator and its operational managers and that this will be managed according to the principles established in the Data Protection Act 1998.
11. I understand that the information contained in this application, any supporting documentation and all
correspondence with the Ethics Committee and its Administrator relating to the application:

- Will be held by the Ethics Committee until at least 3 years after the end of the study
- Will be subject to the provisions of the Freedom of Information Acts and may be disclosed in response to requests made under the Acts except where statutory exemptions apply.
- May be sent by email or other electronic distribution to Ethics Committee members.

Principal Investigator…….. **Date…….**

Supervisor:  **Date…24/3/16…….**

Appendix 2: Ethical Approval Letter (Original Version)

Ethics-fhss@port.ac.uk

06 June 2016

Dear Helen Ody

Study Title:	**The study and application of underwater decomposition from an entomological perspective for the purpose of post-mortem interval estimation**
Ethics Committee reference:	**15/16:29**

Thank you for submitting your documents for ethical review. The Ethics Committee was content to grant a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, revised in the light of any conditions set, subject to the general conditions set out in the attached document.

There is no need to submit any further evidence to the Ethics Committee; the favourable opinion has been granted with the assumption of compliance

The favourable opinion of the EC does not grant permission or approval to undertake the research. Management permission or approval must be obtained from any host organisation, including University of Portsmouth, prior to the start of the study.

Appendix 3: Ethical Approval Letter Following Substantial Amendments

University of Portsmouth
Faculty of Humanities and Social Sciences
Ethics-fhss@port.ac.uk

Substantial Amendment

6th April 2017

Dear Helen Ody,

Study Title:	**The Study and Application of Underwater Decomposition from an Entomological Perspective for the Purpose of Postmortem Interval Estimation**
Ethics Committee reference:	**15/16: 29**

Thank you for submitting your documents for ethical review. The Ethics Committee was content to grant a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, revised in the light of any conditions set, subject to the general conditions set out in the attached document.

The Ethics committee provides a favourable ethical opinion for this substantial amendment

There is no need to submit any further evidence to the Ethics Committee; the favourable opinion has been granted with the assumption of compliance

FORM UPR16
Research Ethics Review Checklist

Please include this completed form as an appendix to your thesis (see the Research Degrees Operational Handbook for more information

Postgraduate Research Student (PGRS) Information			**Student ID:**	UP795241
PGRS Name:	Helen Ody			
Department:	ICJS	**First Supervisor:**	Dr. Katherine Brown	
Start Date: (or progression date for Prof Doc students)	**September 2015**			

Study Mode and Route:	Part-time ☐	MPhil ☐	MD ☐
	Full-time ☒	PhD ☒	Professional Doctorate ☐

Title of Thesis:	
Thesis Word Count: (excluding ancillary data)	

If you are unsure about any of the following, please contact the local representative on your Faculty Ethics Committee for advice. Please note that it is your responsibility to follow the University's Ethics Policy and any relevant University, academic or professional guidelines in the conduct of your study

Although the Ethics Committee may have given your study a favourable opinion, the final responsibility for the ethical conduct of this work lies with the researcher(s).

UKRIO Finished Research Checklist: (If you would like to know more about the checklist, please see your Faculty or Departmental Ethics Committee rep or see the online version of the full checklist at: http://www.ukrio.org/what-we-do/code-of-practice-for-research/)	
a) Have all of your research and findings been reported accurately, honestly and within a reasonable time frame?	YES ☒ NO ☐
b) Have all contributions to knowledge been acknowledged?	YES ☒ NO ☐

c) Have you complied with all agreements relating to intellectual property, publication and authorship?	YES ☒ NO ☐
d) Has your research data been retained in a secure and accessible form and will it remain so for the required duration?	YES ☒ NO ☐
e) Does your research comply with all legal, ethical, and contractual requirements?	YES ☒ NO ☐

Candidate Statement:		
I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s)		
Ethical review number(s) from Faculty Ethics Committee (or from NRES/SCREC):		15/16: 29
If you have *not* submitted your work for ethical review, and/or you have answered 'No' to one or more of questions a) to e), please explain below why this is so:		
N/A		
Signed *(PGRS)*:	Helen Ody	**Date:** 21/09/19

2017 Aquatic Recovery Questionnaire

Welcome

Institute of Criminal Justice Studies

University of Portsmouth

Ravelin House

Museum Road

Portsmouth

PO1 2QQ

Research student: Helen Ody, helen.ody@port.ac.uk

Project supervisor: Dr. Katherine Brown, katherine.brown@port.ac.uk, 023 9284 5247

Head of Department: Dr. Phil Clements, phil.clements@port.ac.uk, 023 9284 5069

Study Title: The study and application of underwater decomposition from an entomological perspective for the purpose of post-mortem interval estimation

Dear Potential Participant

You are invited to participate in a research study entitled “The study and application of underwater decomposition from an entomological perspective for the purpose of post-mortem interval estimation”. You have been identified as a potential participant through your involvement with a relevant organisation. This study is an investigation of

decomposition on land and in water for the purposes of estimating time since death in forensic investigations. We are interested in your experiences, knowledge and awareness of different aspects of the process of recovering human remains from water.

In order to participate, you will be asked to complete this online questionnaire and consent form. Please print a copy of the consent form to retain for yourself. You may also be asked to participate in a verbal (telephone/Skype/face-to-face) interview. Please indicate your consent to be contacted for this purpose by ticking the appropriate box on the consent form. Only participants who have completed the questionnaire will be invited to take part in an interview. If you do not wish to participate in the questionnaire, please close this window now and you will be considered withdrawn from the study.

Your participation is completely voluntary, data will be made anonymous where appropriate, and you may withdraw at any time before completing the questions. Should you wish to withdraw, please simply close the window in your internet browser before reaching the end of the questions. Please note that it is the intention for this research to be published, however all results will be reported anonymously.

Should you have any questions or require any further information about the study, please feel free to contact the research student using the email or postal addresses above.

Lastly, please note that this version of the questionnaire is not aimed at students. If you are a student, we would like to thank you for your interest in taking part, and invite you to exit the questionnaire at this stage.

Thank you for taking the time to read this. Please select "Next" to continue.

Additional Information

About this study

This study uses a mixed methods approach to investigate decomposition and insect succession in different environments (on land and in water) for use in forensic investigations and forensic entomology research. Alongside the practical aspect of the study, questionnaires are being distributed to people in occupations or volunteering roles who have been or may be involved with recovery of human remains or rescue of live persons from water. The questions are designed to explore your experiences, opinions, knowledge and awareness of different aspects of this type of investigation.

What is forensic entomology?

Forensic entomology is the use of insects and other invertebrates during forensic investigations. The most common application of forensic entomology is estimation of time since death (postmortem interval) where human remains have been found an unknown length of time after death. This may be applied to both suspicious and non-suspicious deaths. Typically a forensic entomologist may study aspects such as insect lifecycles in order to estimate postmortem interval. As insects are attracted to human remains very soon after death, they can be used to help provide important information for death scene investigations.

Consent

Please read the following statement of consent carefully.

If you agree to participate, please select the "Agree" option to continue to the questionnaire. Upon completion of the survey you will be provided with an option to print out your responses including the statement of consent. We recommend you print a copy to retain for your records.

If you do not agree to any part of the statement, please select the "Disagree" option and you will be withdrawn from the study.

1. I confirm that I have read and understand the information sheet (on previous page) for this study. I have had the opportunity to consider the information, ask questions (via email or post) and have had these answered satisfactorily.

2. I understand that my participation in the questionnaire is voluntary and that I am free to withdraw at any time up to the point of completing the questions without giving any reason.

3. I understand that data collected during the study may be looked at by individuals from the University of Portsmouth, or from regulatory authorities. I give permission for these individuals to have access to my data.

4. I agree to take part in the study. * *Required*

- ○ Agree
- ○ Disagree

Respondent Demographics

Have you ever attended a death scene in the course of a current or previous job/volunteering/shadowing role? * *Required*

- Yes
- No

Respondent Demographics

Have you ever been involved in the rescue of live persons from water as part of a current or previous job/volunteering/shadowing role?

- Yes
- No

Respondent Demographics

In order to verify that you are an employee or volunteer of one of our target organisations, please select your occupation from the list below. * *Required*

- Police officer (any level)
- Fire officer (any level)
- Forensic entomologist
- RNLI, coastguard or search & rescue volunteer/employee
- CSI or CSM
- Pathologist or mortuary assistant/technician
- Doctor or Paramedic
- Diver (for police)
- Other forensic practitioner
- Other related profession

If you selected Other, please specify:

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RNLI, Coastguard and Search & Rescue Respondent Demographics

Please answer the following questions about you.

Are you:

- Male
- Female
- Other/Prefer not to say

What is your age?

What is your current role and rank/level (if applicable)?

More info

How long have you been in your current role?

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What is your total length of service with the RNLI, Coastguard, or Search & Rescue organisation?

RNLI, Coastguard and Search & Rescue

Please answer the following questions, following any instructions provided.

If you are unable or do not wish to answer any of the questions, please simply leave the box empty and move on to the next question.

Please state which region you currently work or volunteer in.

- North East
- North West
- Yorkshire and the Humber
- East of England
- West Midlands
- East Midlands
- London
- South of England
- South East
- South West
- Northern Ireland
- Scotland
- Wales

Please state the total number of callouts you attended in the past year. An estimate is acceptable if you do not know the exact number.

Is this typical of the number of callouts you would attend in a year?

- Yes
- No
- Not sure or N/A

Of the total number of callouts you attended in the past year, how many resulted in the rescue of living persons who had been in difficulty in water? Again, please estimate if you do not know the exact number.

And how many (if any) of the callouts you attended in the last year involved any fatalities or recovery of human remains from water?

Thinking about your entire length of service with the RNLI, Coastguard or Search & Rescue organisation, how many callouts have you attended which resulted in the rescue of living persons who had been in difficulty in water? Please estimate if you do not know the exact number.

And how many (if any) of the callouts you have attended in your entire length of service involved any fatalities or recovery of human remains from water?

Have you ever had any contact with any forensic personnel (e.g. CSI or forensic specialist) in the course of your work or volunteering role?

- Yes
- No
- Not sure or N/A

If yes, was any of this contact with a forensic entomologist?

- Yes
- No
- Not sure or N/A

Using the scale below, please rate your personal awareness of forensic entomology as an investigative tool:

- I am not aware of the existence of forensic entomology or do not understand the meaning of the phrase
- I have heard of forensic entomology but I know little or nothing about what it involves
- I have a moderate understanding of forensic entomology and am aware of some of the techniques, procedures, and applications of the discipline
- I have a good understanding of forensic entomology and am aware of many of the techniques, procedures, and applications of the discipline
- I have a thorough and comprehensive understanding of forensic entomology, including the techniques, procedures, and applications of the discipline

We are also interested in collecting information relating to the geographic location of any rescues/recoveries. If you have the data available and would be willing to provide it, please enter your contact details (name, email address, telephone number) in the box below. If you do not have the data or do not wish to provide it, simply leave the box empty. Your contact details will not be used for any other purpose, nor will any of your details be in any way published, shared, or distributed. Any data which is published in any format will be anonymised.

Forensic Entomologist Respondent Demographics

Please answer the following questions about you.

Are you:

- Male
- Female
- Other/Prefer not to say

What is your age?

What is your current role and rank/level (if applicable)?

More info

Please enter the start date of your first forensic entomology position.

Dates need to be in the format 'DD/MM/YYYY', for example 27/03/1980.

(dd/mm/yyyy)

Please enter the start date of your first forensic entomology case.

Dates need to be in the format 'DD/MM/YYYY', for example 27/03/1980.

(dd/mm/yyyy)

Please enter the start date of your current forensic entomology position (if applicable).

Dates need to be in the format 'DD/MM/YYYY', for example 27/03/1980.

(dd/mm/yyyy)

Forensic Entomologists

Please answer the following questions following any instructions provided.

If you are unable or do not wish to answer any of the questions, please simply leave the box empty and move on to the next question.

Please state the total number of forensic cases you were involved in during the past year. An estimate is acceptable if you do not know the exact number.

Thinking about your answer to the previous question, is this typical of the number of cases you would be involved in during a one year period?

- Yes
- No
- Not sure or N/A

How many forensic cases have you been involved in during your entire forensic entomology career? Please estimate if you do not know the exact number.

Think about your answer to the question about how many forensic cases you were involved in during your entire career. If you are aware of the circumstances of the cases, please state how many occurred outdoors on land (do not include any which were inside any kind of building, vehicle, or other enclosed structure). If you do not know

enough case details to be able to provide this information, simply leave the box empty.

If you are aware of the circumstances of the cases discussed in your previous answer, please give details of the type of insect evidence recovered. If you are unable to or do not wish to share any details, please skip this question. Please select all that apply.

- [ ] Dipteran eggs
- [ ] Dipteran larvae
- [ ] Dipteran pupae
- [ ] Dipteran puparia/pupal casings
- [ ] Adult diptera
- [ ] Coleoptera (terrestrial) - any life stage
- [ ] Lepidoptera - any life stage
- [ ] Hymenoptera - any life stage
- [ ] Any aquatic species (for example Ephemeroptera, Odonata, Trichoptera larvae, aquatic Coleoptera)
- [ ] Other insect evidence not listed (please state)
- [ ] Other

If you selected Other, please specify:

Thinking about the previous question, did you recover the evidence personally, or was it recovered by a third party?

- ○ Recovered personally

- ○ Recovered by a third party
- ○ N/A

If the evidence was recovered by a third party, please state who this evidence was recovered by (e.g. a CSI). If you do not know, please leave the box blank.

If you are aware of the circumstances, please state how many cases you have ever been involved in that occurred outdoors in water (including ponds, rivers, lakes, streams, canals, reservoirs, the ocean, outdoor swimming pools, and any other water sources). If you do not know enough case details to be able to provide this information, simply leave the box empty.

If you are aware of the circumstances of the cases discussed in your previous answer, please give details of the type of insect evidence recovered. If you are unable to or do not wish to share any details, please skip this question. Please select all that apply.

- [ ] Dipteran eggs
- [ ] Dipteran larvae
- [ ] Dipteran pupae
- [ ] Dipteran puparia/pupal casings
- [ ] Adult diptera
- [ ] Coleoptera (terrestrial) - any life stage
- [ ] Lepidoptera - any life stage
- [ ] Hymenoptera - any life stage
- [ ] Any aquatic species (for example Ephemeroptera, Odonata, Trichoptera larvae, aquatic Coleoptera)

- [ ] Other insect evidence not listed (please state)
- [ ] Other

If you selected Other, please specify:

Thinking about the previous question, did you recover the evidence personally, or was it recovered by a third party?

- ○ Recovered personally
- ○ Recovered by a third party
- ○ N/A

If the evidence was recovered by a third party, please state who this evidence was recovered by (e.g. a CSI). If you do not know, please leave the box blank.

If you have ever received evidence to work on that you did not personally recover, please rate the quality of the evidence using the scale below.

- ○ N/A - I have never received evidence recovered by a third party.
- ○ Excellent - all specimens relevant and collected/packaged correctly with no damage.
- ○ Good - most specimens relevant and mostly collected/packaged correctly. Minimal damage present.
- ○ Adequate - few specimens relevant or largely collected/packaged incorrectly resulting in moderate damage; some specimens still usable.

- ○ Poor - most specimens irrelevant or incorrectly collected/packaged resulting in extensive damage to specimens. Most specimens unusable.
- ○ Very poor - No relevant specimens or all specimens damaged beyond use.

Please state the country or countries where you were practicing when you worked on the cases discussed above.

From the list below, please select your preferred method(s) for determination of minimum post-mortem interval (mPMI) [or minimum period of insect activity (mPIA)] using insect evidence. Select as many as apply.

- [ ] Insect rearing
- [ ] Age estimation from Dipteran immature stages
- [ ] Isomorphen/Isomegalen diagrams
- [ ] Insect life cycle development data
- [ ] Digital or computerised methods e.g. ForenSeek
- [ ] Insect colonisation/succession patterns
- [ ] Insect morphology
- [ ] DNA species identification
- [ ] Other - please describe
- [ ] Other

If you selected Other, please specify:

Forensic Entomologists

During your career in forensic entomology, are you aware of your evidence ever being used in subsequent court cases?

More info

- Yes
- No

If yes, please state the number of cases you have worked on where your evidence was used in a court case. Please estimate if you do not know the exact number.

Using the scale below, please indicate your opinion on how well forensic entomology is being used as an investigative tool:

Please don't select more than 1 answer(s) per row.

	Badly under-used	Somewhat under-used	Used an acceptable amount	Well used	Used to its full potential
Please select	☐	☐	☐	☐	☐

Police/Fire Officer/CSI/Other related profession - Respondent Demographics

Please answer the following questions about you.

Are you:

- Male
- Female
- Other/Prefer not to say

What is your age?

What is your current role and rank (if applicable)?

More info

How long have you been in your current role?

What is your total length of service?

Police Officer/Fire Officer/CSI/Other related profession

Please answer the following questions using any instructions provided.

If you are unable or do not wish to answer any of the questions, please simply leave the box empty and move on to the next question.

Please state which region you currently work in.

- North East
- North West
- Yorkshire and the Humber
- East of England
- West Midlands
- East Midlands
- London
- South of England
- South East
- South West
- Northern Ireland
- Scotland
- Wales

Please state the number of death scenes you attended in the past year. An estimate is acceptable if you do not know the exact number. If you do not attend any death scenes, simply enter 0 into the box provided. For this question, please consider all types of death scene regardless of circumstances or location.

Thinking about your answer to the previous question, is this typical of the number of death scenes you would attend in one year?

- Yes
- No
- Not sure or N/A

Thinking back to your answer about how many death scenes you attended in the past year, how many were outdoors on land (do not include any which were inside any kind of building, vehicle, or other enclosed structure)? An estimate is acceptable if you do not know the exact number.

Of the total number of death scenes you attended in the past year, how many were outdoors in water (including ponds, rivers, lakes, streams, canals, reservoirs, the ocean, outdoor swimming pools, and any other water sources)? Please estimate if you do not know the exact answer.

How many death scenes have you attended in your entire career? Please estimate if you do not know the exact number, and please consider all types of death scene regardless of circumstances or location.

Of the total number of death scenes you have attended in your entire career, how many were outdoors on land (do not include any which were inside any kind of building, vehicle, or other enclosed structure)? An estimate is acceptable if you do not know the exact number.

Of the total number of death scenes you attended in your entire career, how many were outdoors in water (including ponds, rivers, lakes, streams, canals, reservoirs, the ocean, outdoor swimming pools, and any other water sources)? Please estimate if you do not know the exact answer.

Police Officer/Fire Officer/CSI/Other related profession

Using the scale below, please rate your personal awareness of forensic entomology as an investigative tool:

- I am not aware of the existence of forensic entomology or do not understand the meaning of the phrase
- I have heard of forensic entomology but I know little or nothing about what it involves
- I have a moderate understanding of forensic entomology and am aware of some of the techniques, procedures, and applications of the discipline
- I have a good understanding of forensic entomology and am aware of many of the techniques, procedures, and applications of the discipline
- I have a thorough and comprehensive understanding of forensic entomology, including the techniques, procedures, and applications of the discipline

Thinking back to the death scenes you attended in the past year, please estimate how many scenes involved the collection of insect evidence.

Thinking back to the death scenes you attended in the past year, please estimate how many scenes involved insect evidence which was observed but NOT collected.

Of the total number of death scenes you have attended in your entire career, please estimate how many scenes involved the collection of insect evidence.

Thinking back to the death scenes you have attended in your entire career, please estimate how many scenes involved insect evidence which was observed but NOT collected.

Have you ever personally collected any entomological evidence?

More info

- Yes
- No

Have you ever been responsible for contacting/engaging with a forensic entomologist as part of a case?

- Yes
- No

If you are or have ever been responsible for engaging with an entomologist for cases, do you have a specific point of contact or a specific entomologist that you use?

- Yes
- No

- N/A - I am not responsible for this

Using the scale below, please indicate your opinion on how well forensic entomology is being used as an investigative tool:

Please don't select more than 1 answer(s) per row.

	Badly under-used	Somewhat under-used	Used an acceptable amount	Well used	Used to its full potential	Unsure/Not applicable
Please select	☐	☐	☐	☐	☐	☐

Medical Professionals - Respondent Demographics

Please answer the following questions about you.

Are you:

- Male
- Female
- Other/Prefer not to say

What is your age?

What is your current role and rank/level (if applicable)?

More info

How long have you been in your current role?

What is the total length of your career in the medical profession?

Medical Professionals

Please answer the following questions using any instructions provided.

If you are unable or do not wish to answer any of the questions, please simply leave the box empty and move on to the next question.

Please state which region you currently work in.

- North East
- North West
- Yorkshire and the Humber
- East of England
- West Midlands
- East Midlands
- London
- South of England
- South East
- South West
- Northern Ireland
- Scotland
- Wales

Please state the total number of death scenes you attended in the past year. An estimate is acceptable if you do not know the exact number. If you do not attend any death scenes, simply enter 0 into the box provided. For this question, please consider all types of death scene regardless of circumstances or location.

Thinking about your answer to the previous question, is this typical of the number of death scenes you would attend in a one year period?

- Yes
- No
- Not sure or N/A

Thinking back to your answer about how many death scenes you attended in the past year, how many were outdoors on land (do not include any which were inside any kind of building, vehicle, or other enclosed structure)? An estimate is acceptable if you do not know the exact number.

Of the total number of death scenes you attended in the past year, how many were outdoors in water (including ponds, rivers, lakes, streams, canals, reservoirs, the ocean, outdoor swimming pools, and any other water sources)? Please estimate if you do not know the exact answer.

How many death scenes have you attended during your entire career? Again, please estimate if you do not know the exact number, and for this question please consider all types of death scene regardless of circumstances or location.

Of the total number of death scenes you have attended in your entire career, how many were outdoors on land (do not include any which were inside any kind of building, vehicle, or other enclosed structure)? An estimate is acceptable if you do not know the exact number.

Of the total number of death scenes you attended in your entire career, how many were outdoors in water (including ponds, rivers, lakes, streams, canals, reservoirs, the ocean, outdoor swimming pools, and any other water sources)? Please estimate if you do not know the exact answer.

Medical Professionals

Using the scale below, please rate your personal awareness of forensic entomology as an investigative tool:

- I am not aware of the existence of forensic entomology or do not understand the meaning of the phrase
- I have heard of forensic entomology but I know little or nothing about what it involves
- I have a moderate understanding of forensic entomology and am aware of some of the techniques, procedures, and applications of the discipline
- I have a good understanding of forensic entomology and am aware of many of the techniques, procedures, and applications of the discipline
- I have a thorough and comprehensive understanding of forensic entomology, including the techniques, procedures, and applications of the discipline

Have you ever observed any entomological evidence on human remains during the course of your work?

- Yes
- No
- Not sure or N/A

Have you ever collected any entomological evidence from human remains during the course of your work?

- Yes
- No
- Not sure or N/A

Pathologists/Mortuary assistants - Respondent Demographics

Please answer the following questions about you.

Are you:

- Male
- Female
- Other/Prefer not to say

What is your age?

What is your current role and rank/level (if applicable)?

More info

How long have you been in your current role?

What is your total length of service?

Pathologists/Mortuary assistants

Please answer the following questions using any instructions provided.

If you are unable or do not wish to answer any of the questions, please simply leave the box empty and move on to the next question.

Please state which region you currently work in.

- North East
- North West
- Yorkshire and the Humber
- East of England
- West Midlands
- East Midlands
- London
- South of England
- South East
- South West
- Northern Ireland
- Scotland
- Wales

Please state the total number of cadavers you have examined (in the mortuary, in the home or at a death scene) in the past year. An estimate is acceptable if you do not know the exact number. If you did not examine any cadavers, simply enter 0 into the box provided.

Thinking about your answer to the previous question, is this typical of the number of cadavers you would examine in a one year period?

- Yes
- No
- Not sure or N/A

If you are aware of the circumstances, please state how many of the cadavers you examined in the last year were found outdoors on land (do not include any which were inside any kind of building, vehicle, or other enclosed structure)? An estimate is acceptable if you do not know the exact number.

If you are aware of the circumstances, how many of the total number of cadavers you examined in the past year were found outdoors in water (including ponds, rivers, lakes, streams, canals, reservoirs, the ocean, outdoor swimming pools, and any other water sources)? Please estimate if you do not know the exact answer.

How many cadavers have you examined during your entire career? Again, please estimate if you do not know the exact number.

If you are aware of the circumstances, how many of the total number of cadavers you

have examined in your entire career were found outdoors on land (do not include any which were inside any kind of building, vehicle, or other enclosed structure)? An estimate is acceptable if you do not know the exact number.

If you are aware of the circumstances, how many of the total number of cadavers you have examined in your entire career were outdoors in water (including ponds, rivers, lakes, streams, canals, reservoirs, the ocean, outdoor swimming pools, and any other water sources)? Please estimate if you do not know the exact answer.

Pathologists/mortuary assistants

Using the scale below, please rate your personal awareness of forensic entomology as an investigative tool:

- I am not aware of the existence of forensic entomology or do not understand the meaning of the phrase
- I have heard of forensic entomology but I know little or nothing about what it involves
- I have a moderate understanding of forensic entomology and am aware of some of the techniques, procedures, and applications of the discipline
- I have a good understanding of forensic entomology and am aware of many of the techniques, procedures, and applications of the discipline
- I have a thorough and comprehensive understanding of forensic entomology, including the techniques, procedures, and applications of the discipline

Have you ever observed any entomological evidence on human remains during the course of your work?

- Yes
- No
- Not sure or N/A

Have you ever collected any entomological evidence from human remains during the course of your work?

- Yes
- No
- Not sure or N/A

If you are aware of the circumstances, please give details of the type of insect evidence recovered. If you are unable to or do not wish to share any details, please skip this question. Please select all that apply.

- [ ] Fly eggs
- [ ] Fly larvae
- [ ] Fly pupae
- [ ] Adult flies
- [ ] Beetles
- [ ] Other adult insects - please state if possible
- [ ] Other immature insects - please state if possible

If you selected Other, please specify:

Have you ever worked with a forensic entomologist to establish time since death?

- ( ) Yes
- ( ) No

Interview Consent

In some cases we may wish to contact respondents in order to take part in a verbal interview (face-to-face/Skype/telephone).

Please read the following statement of consent carefully and indicate for each section whether you agree.

I agree to being contacted by the researcher in order to arrange a verbal interview (telephone/Skype/face-to-face) at a mutually convenient time.

- ( ) Agree
- ( ) Disagree

If you agree to being contacted to arrange an interview, please provide your contact details (name/email address/telephone number) below.

I understand that my participation in the interview is voluntary and that I am free to withdraw at any time up to the point of data analysis (by December 2018) without giving any reason.

- ( ) Agree
- ( ) Disagree

I understand that data collected during the study may be looked at by individuals from the University of Portsmouth, or from regulatory authorities. I give permission for these individuals to have access to my data.

- ○ Agree
- ○ Disagree

I agree to the audio from my interview being recorded.

- ○ Agree
- ○ Disagree

I agree to being quoted verbatim.

- ○ Agree
- ○ Disagree

Finish

Please select "Finish" to end the questionnaire and have your responses recorded.

Thank you

Thank you for taking part in this questionnaire.

You may now close this page.

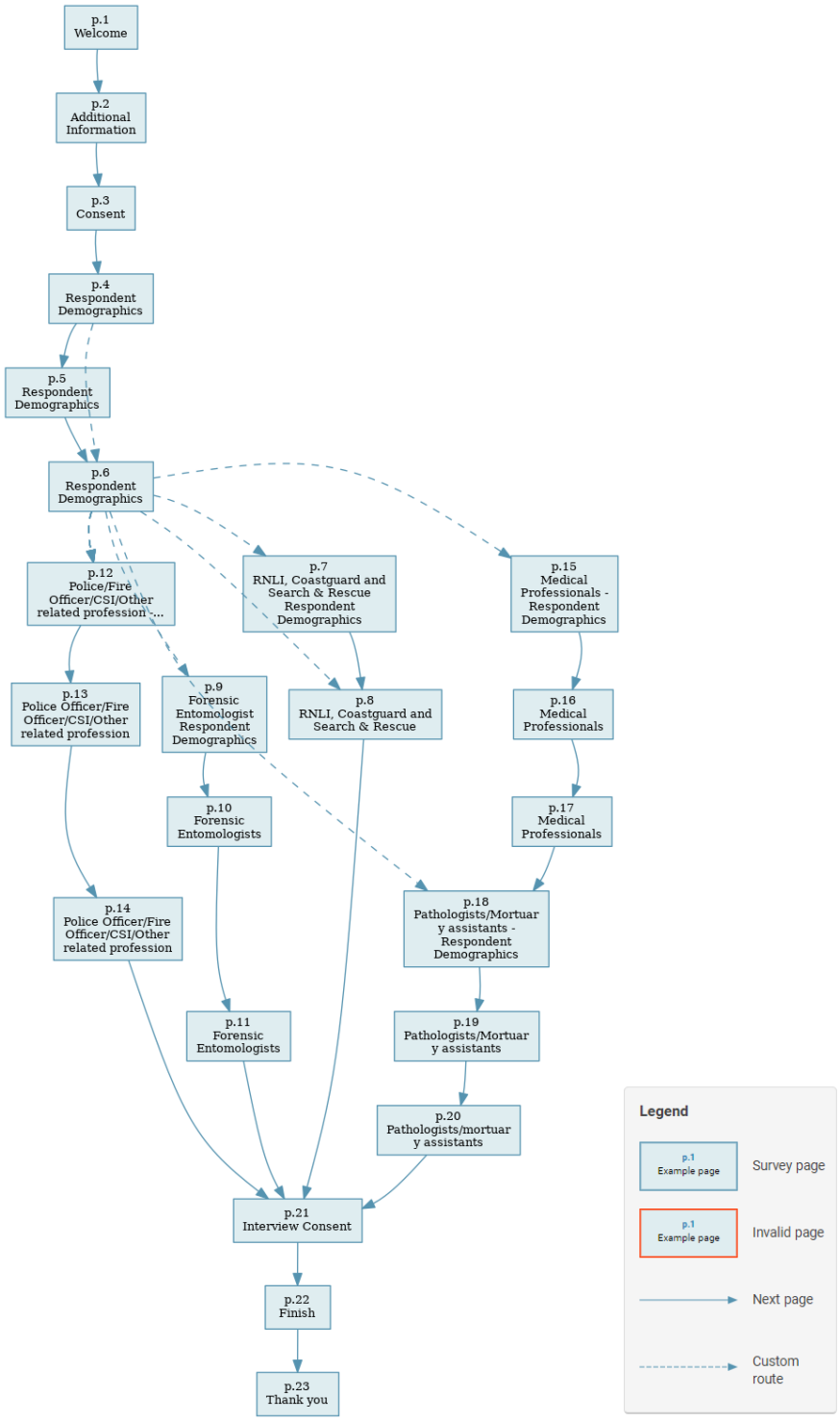


Figure 68: Survey map showing routing of participants through questions

Appendix 6: Maps of Study Sites for the Project

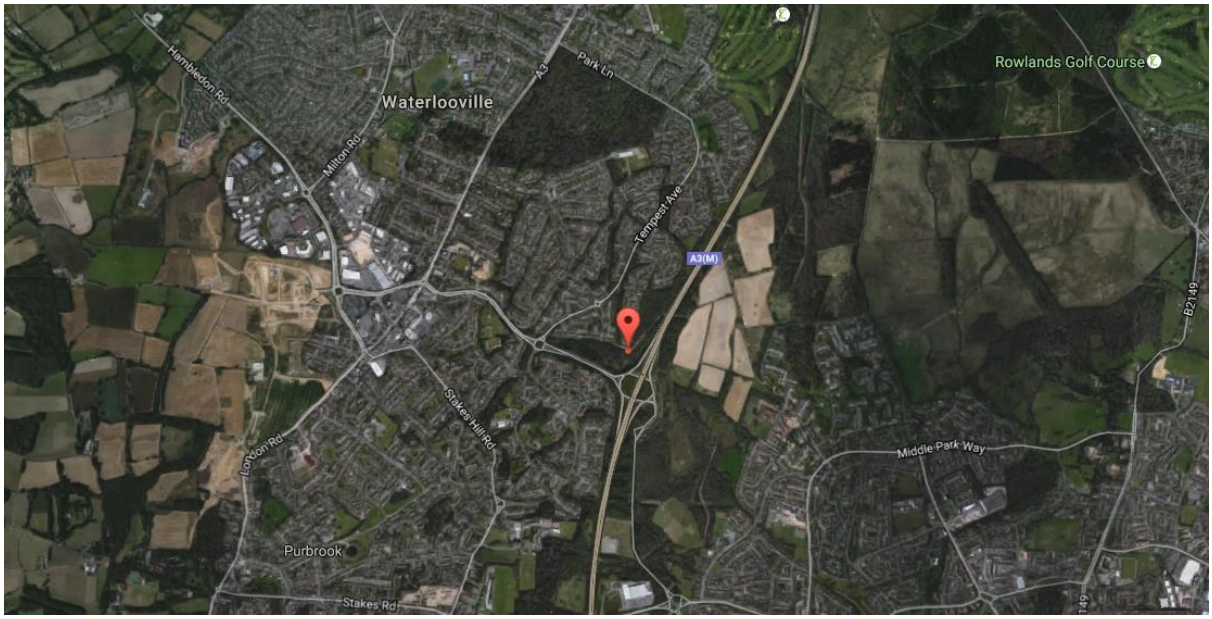


Figure 69: Map showing location of freshwater stream used to collect water for pilot study (Chapter 5)

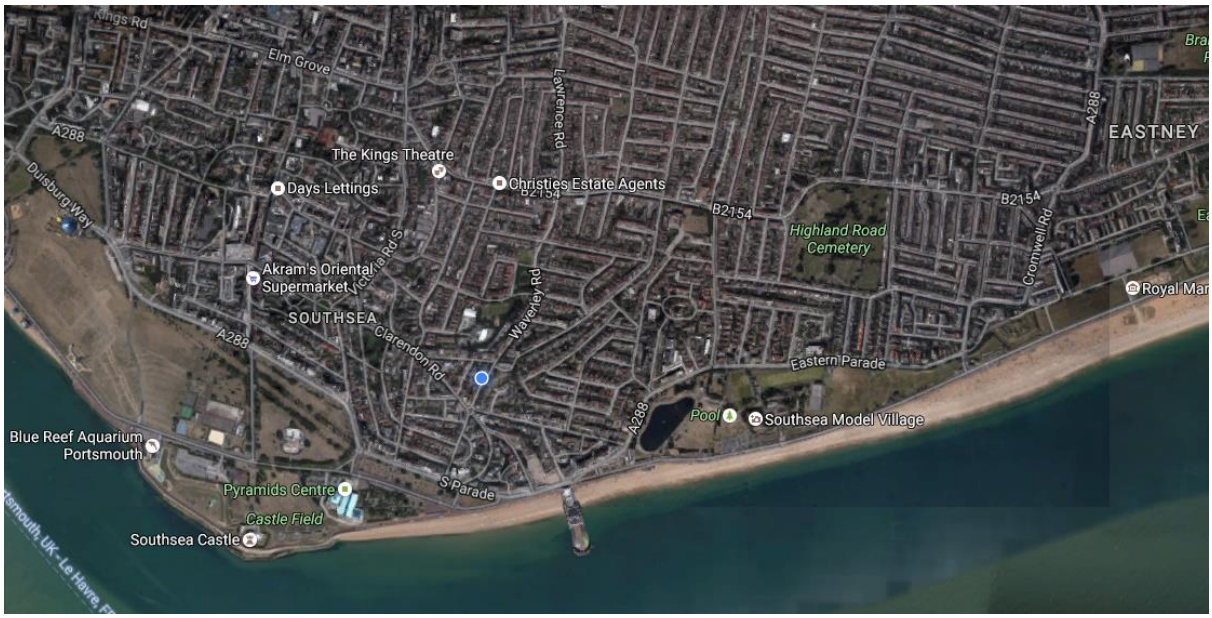


Figure 70: Map showing south end of Portsea Island where sea water was collected for pilot study (Chapter 5)

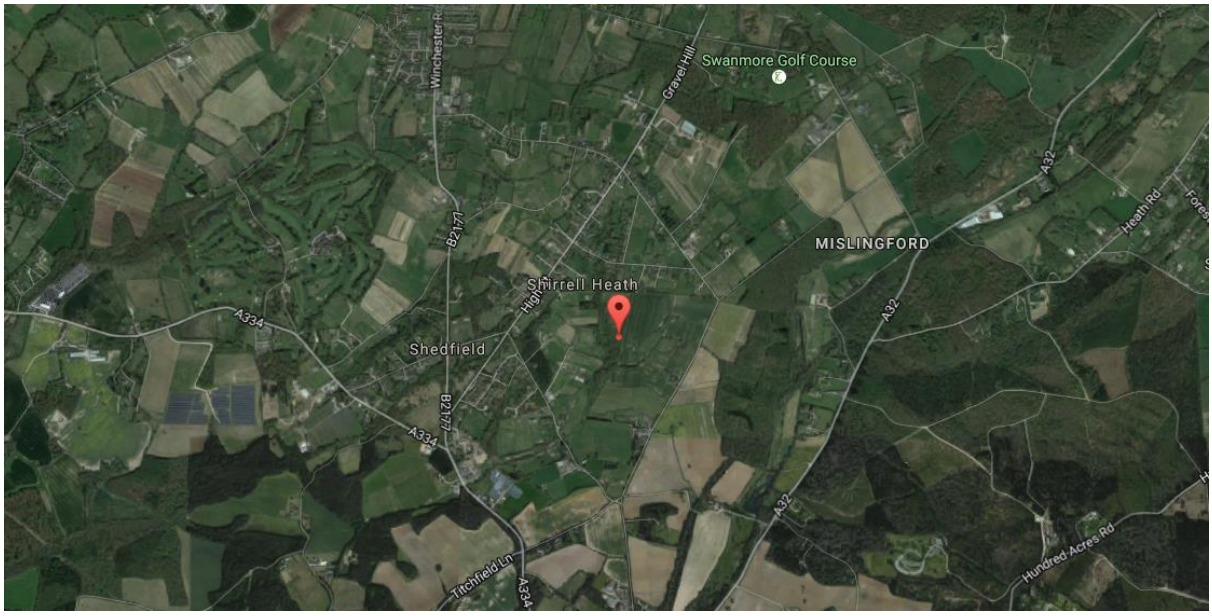


Figure 71: Map showing location of woodland site near Wickham which was used for the freshwater field study (Chapter 6)

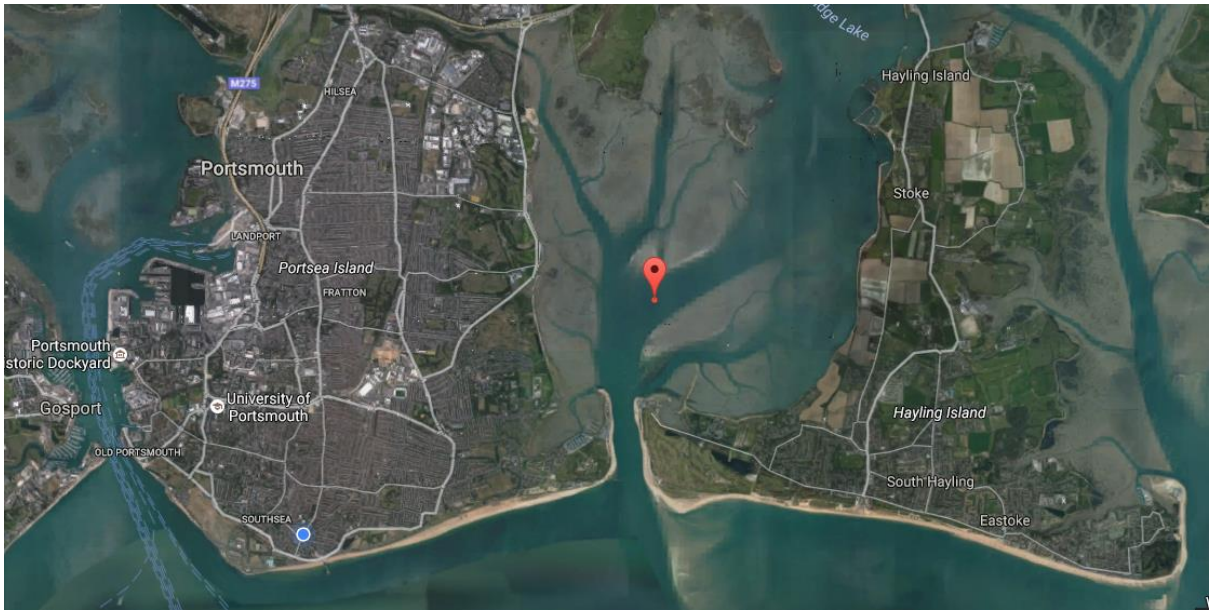
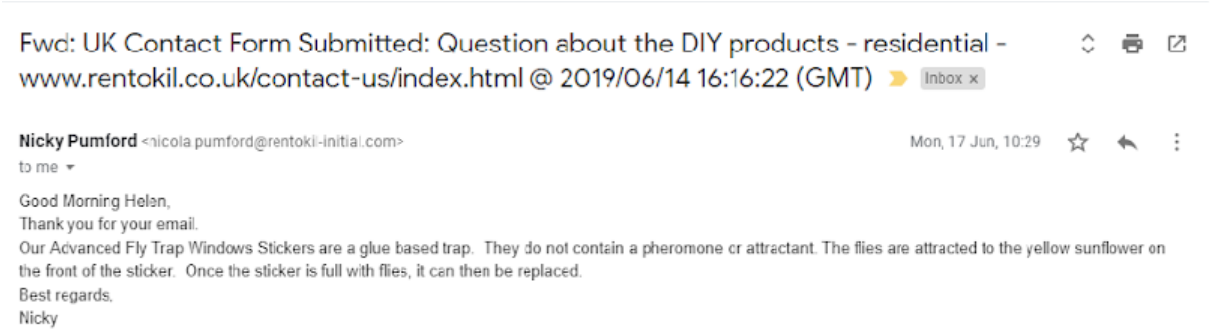


Figure 72: Map showing location of University of Portsmouth Institute of Marine Sciences' research raft used for harbour studies

Appendix 7: Email confirming lack of attractants in Rentokil Insect Sticky Traps



Fwd: UK Contact Form Submitted: Question about the DIY products - residential - www.rentokil.co.uk/contact-us/index.html @ 2019/06/14 16:16:22 (GMT) Inbox ×

Nicky Pumford <nicola.pumford@rentokil-initial.com> Mon, 17 Jun, 10:29

to me

Good Morning Helen,
Thank you for your email.
Our Advanced Fly Trap Windows Stickers are a glue based trap. They do not contain a pheromone or attractant. The flies are attracted to the yellow sunflower on the front of the sticker. Once the sticker is full with flies, it can then be replaced.
Best regards,
Nicky

Appendix 8: Introduction to Study into Survival of Submerged Blow Fly Eggs

Introduction

This experiment was designed to investigate survival of blow fly eggs when submerged in water for different periods of time. The study was designed in response to observations made during the pilot study reported in this thesis (Chapter 5) where blow fly eggs laid on the surface of floating remains were seen to be temporarily submerged in shallow water due to water lapping over the surface of the remains. While blow fly eggs are laid in clumps of approximately 180 (Erzinçlioğlu, 1996) it was noted that the partially submerged eggs would sometimes become separated from one another, and as such the experiment was designed to investigate survival of both eggs in clumps and eggs which were separated from each other.

Materials and Method

Approximately twenty freshly laid *C. vicina* eggs were submitted to each of six treatments in order to test the ability of the eggs to survive after submersion in water for different lengths of time (table 1). A non-submerged treatment (i.e. with fully dry eggs) was used as a control, a wet but not submerged treatment was designed to mimic eggs which may have become wet from rainfall or contact with wet clothing, and the third and final treatment fully submerged the eggs in tap water.

Table 12: Conditions of each treatment used to test survival of C. vicina eggs in water

Treatment Number	Treatment Type	Egg Arrangement on Liver
1	Non-submerged control	Clump
2	Non-submerged control	Spread out
3	Wet but not submerged	Clump, covered with damp tissue paper
4	Wet but not submerged	Spread, covered with damp tissue paper
5	Fully submerged	Clump, fully covered with tap water
6	Fully submerged	Spread out, fully covered with tap water

Each treatment ran simultaneously for 30 minutes in the IGC which was regulated at 22^{o}C. 25 g + /- 0.5 g porcine liver was used in each case (fig. 10). After this the liver with the eggs was removed and placed back into the IGC for rearing. The resulting larvae were provided with fresh porcine liver *ad libitum* and the number of pupae reared was recorded. Finally, the number of adults in each case was recorded.

Figure 73: Each treatment ran simultaneously for 30 minutes inside the IGC

Results

Currently, only one set of these experiments has been completed, in which eggs were submerged for 30 minutes as described above. However, the method seems to work correctly and the results are summarised in table 5.

Table 13: Preliminary results for 30 minute submersion treatment on C. vicina eggs

Treatment time	**Treatment type**	**No. of pupae reared**	**No. of adults reared**	**Time to pupation**
30 mins	Control - Non-submerged (spread)	5	5	9 days
	Control -Non-submerged (clump)	16	14 (plus 1 partially emerged)	
	Damp (spread)	8	6	
	Damp (clump)	15	12	
	Fully submerged (spread)	2	2	
	Fully submerged (clump)	0	0	

Discussion

At this time, no further experimentation has been carried out, however in a future version of this study, this protocol will be repeated followed by the treatments being run for increasing lengths of time (1hr, 2hrs, 4hrs etc) until the survival of the eggs reaches 0% in the fully submerged treatments.

In this case, only *C. vicina* eggs were tested, however this experiment can be repeated with other species of blow fly that are known to colonise remains in the UK.

Appendix 9: Harbour Study GoPro™ Images (Chapter 5: A Preliminary Investigation of Faunal Colonisation of Remains in Open Water)

Figure 74: A variety of fauna on and around the modified crayfish pot in method 1b

Figure 75: A crab seen on the modified crayfish pot in method 1b

Figure 76: Crabs on the outside of the modified crayfish pot in method 1b

Figure 77: Crabs seen on the outside of the modified crayfish pot in method 1b

Figure 78: A whelk seen on the outside of the crayfish pot in method 1b (indicated by red circle)

Figure 79: Crabs seen on the outside of the crayfish pot in method 1b

Figure 80: Various fauna seen on the outside of the crayfish pot in method 1b

Figure 81: Various fauna on and around the crayfish pot in method 1b

Figure 82: Fully skeletonised remains of the piglet carcass inside the modified crayfish pot in method 1b

Figure 83: Crab and a number of fish seen on and around the modified crayfish pot in method 1b

Figure 84: Several crabs on the outside of the modified crayfish pot in method 1b. The skeletonised remains of the piglet carcass can be seen inside.

Figure 85: Whelks seen inside the lobster pot in method 3 (first repeat)

Figure 86: Crab and several whelks seen on and around the remains in the lobster pot in method 3 (first repeat)

Figure 87: Several crabs seen on and around the remains in the lobster pot in method 3 (first repeat)

Figure 88: Crabs feeding on the piglet remains in the lobster pot in method 3 (first repeat)

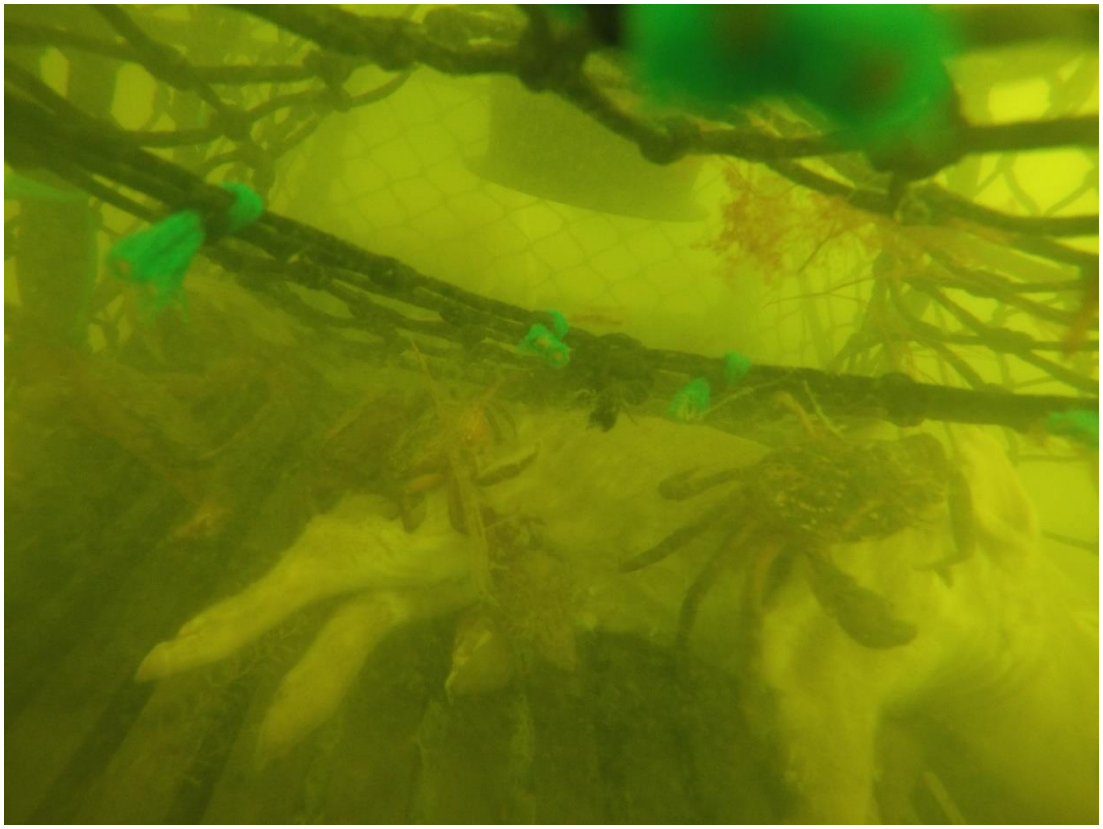


Figure 89: Crabs feeding on the piglet remains in the lobster pot in method 3 (first repeat)

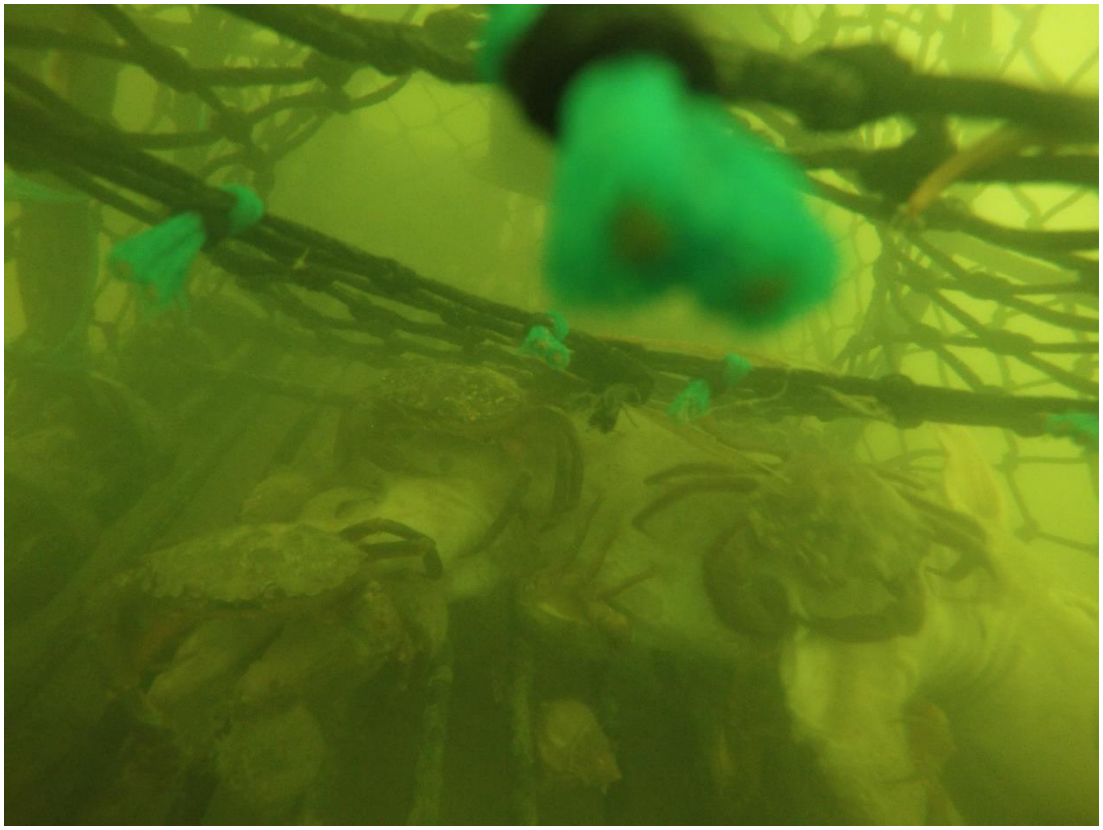


Figure 90: Crabs feeding on the piglet carcass in the lobster pot in method 3 (first repeat)

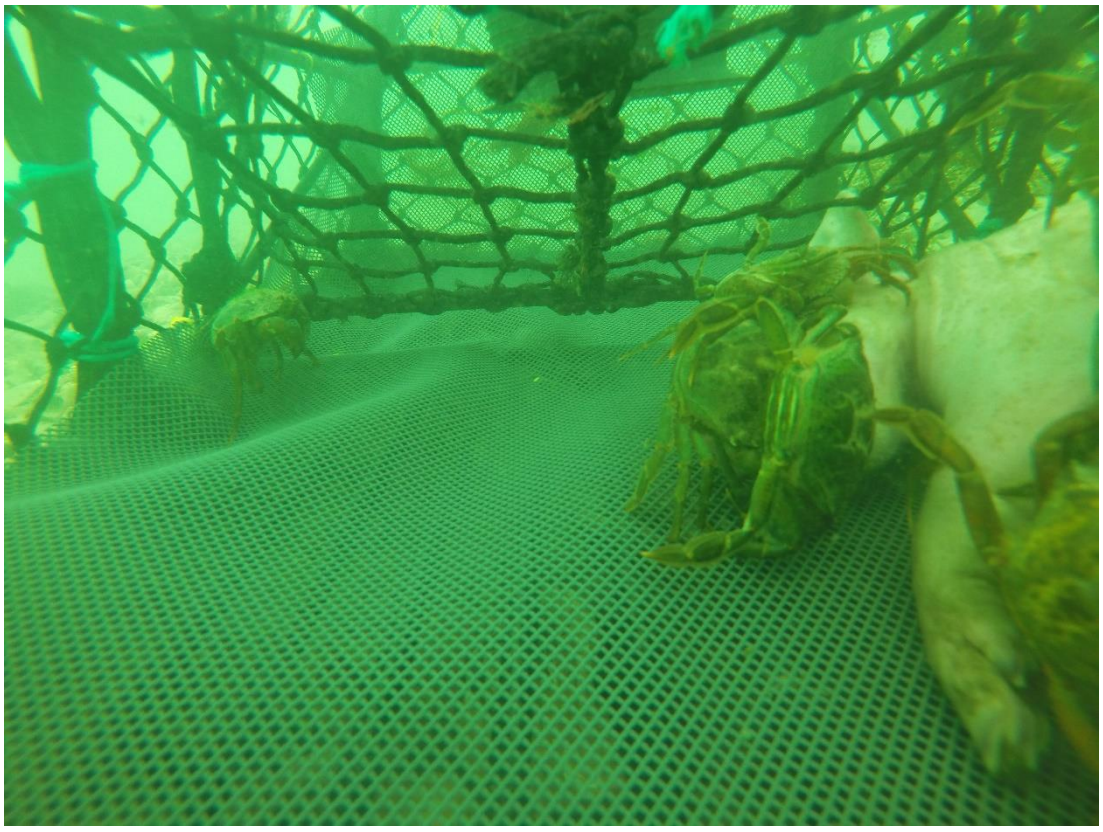


Figure 91: Crabs feeding on the head of the piglet in method 3 (second repeat)

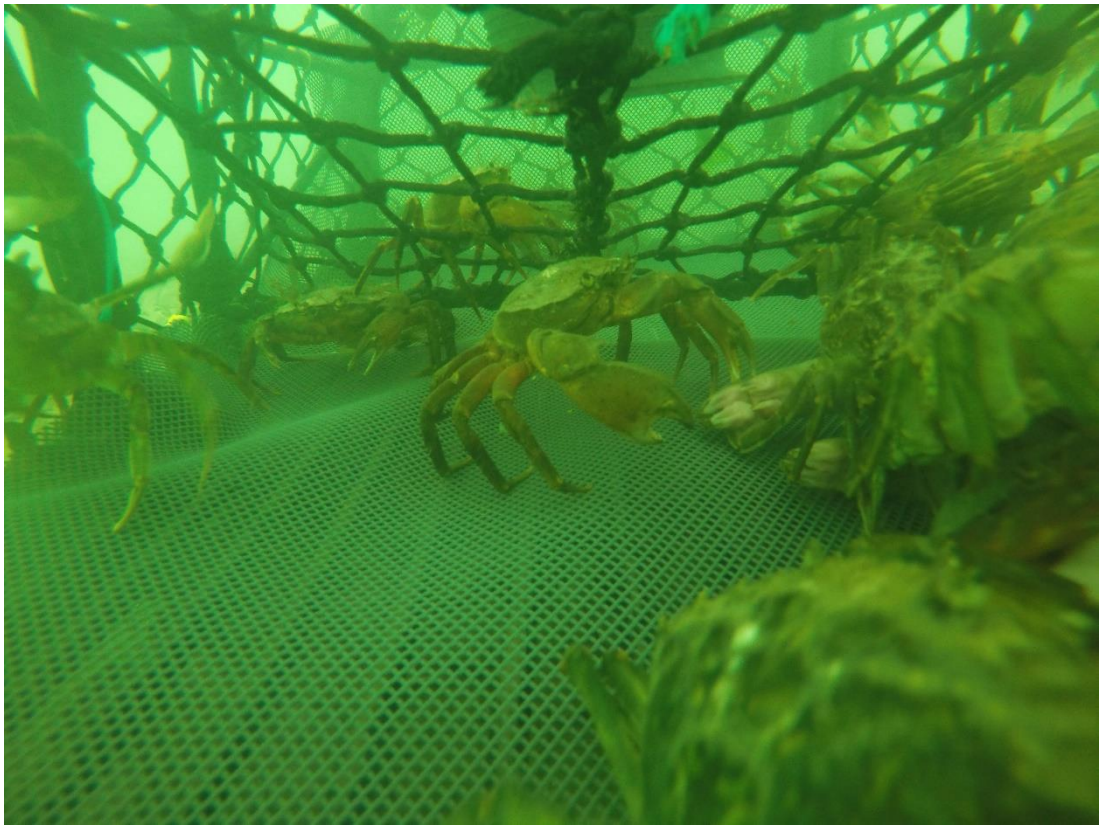


Figure 92: Numerous crabs seen inside the lobster pot in method 3 (second repeat)

Figure 93: Image showing crabs on and around piglet remains inside lobster pot, including a leg which has been at least partially removed (method 3, second repeat)

Figure 94: Numerous crabs on and around the piglet carcass inside the lobster pot in method 3 (second repeat)

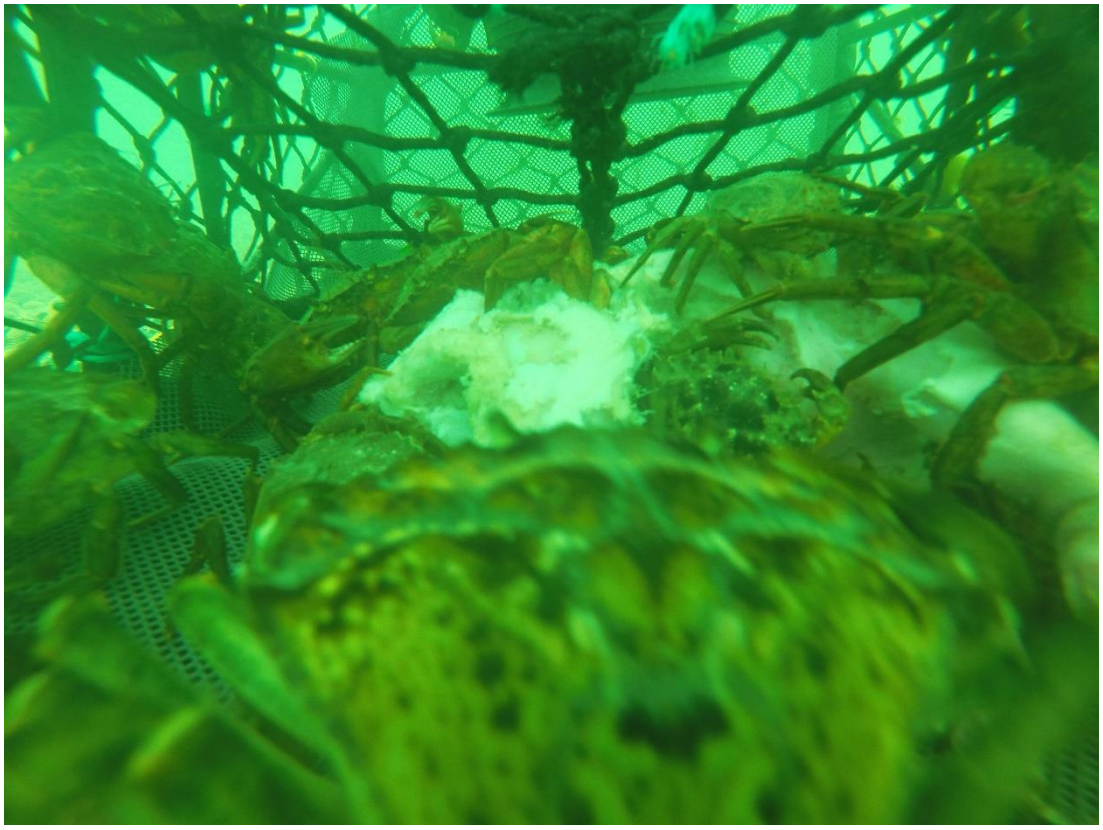


Figure 95: Crabs feeding on the piglet carcass inside the lobster pot in method 3 (second repeat)

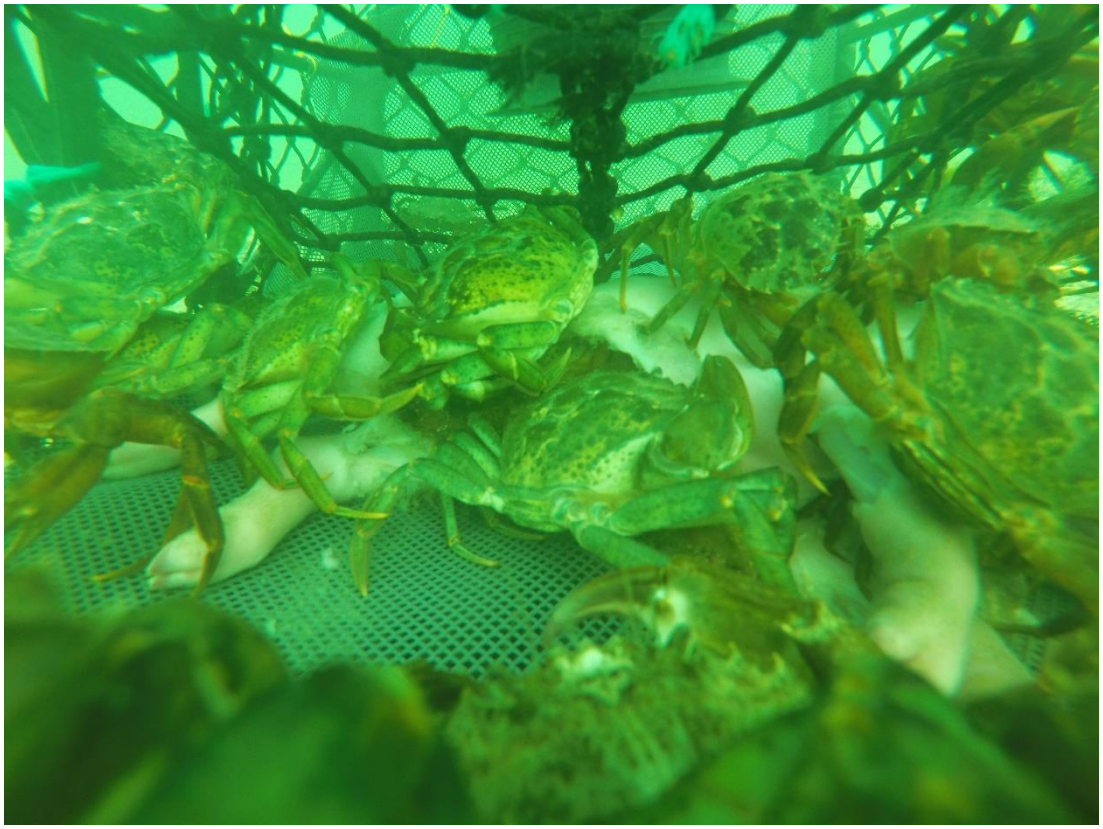


Figure 96: Crabs seen feeding inside an opening in the abdomen of the piglet carcass in method 3 (second repeat)

Figure 97: View from the GoPro™ camera of the inside of the lobster pot at the end of the second repeat of method 3

Appendix 10: Glossary

Abiotic factors'	Physical rather than biological, as in 'abiotic
Adipocere	A waxy substance formed during the decomposition process, especially in wet environments
Ante-mortem	Before death
Anthropology	The study of humans, human behaviour, and human society. When applied to forensic investigation this refers to the analysis of human remains for legal purposes, including establishing identity of the deceased.
Benthic	Relating to the ecological region at the lowest level of a body of water
Diatoms	Single-celled algae which can be found in soils and aquatic environments worldwide
Disarticulation	When applied to skeletal remains, this refers to the separation of bones that occurs as the soft tissue decomposes
Entomology arthropods.	The study of insects. May also include study of
Epinecrotic	Refers to organisms (usually prokaryotes, protists and fungi) present on the surface of decomposing remains (Guo et al., 2016; Pechal et al., n.d.)
Geophysics	The mathematical and physical study of the Earth's internal structure and dynamics (*BSc Geophysics | The University of Edinburgh*, n.d.)
Gram-positive	Refers to bacteria that give a positive result in the Gram stain test, a method used to group

	bacteria into categories based on the structure of their cell wall
Hydrogeophysics	The use of geophysical methods for characterising subsurface features, hydrogeological properties, and processes relevant to soil and groundwater processes (Binley et al., 2010)
Limnology	The study of fauna of inland waters (Wetzel, 2001)
Medicolegal	Pertaining to both medicine and law
Necrophagous	Relating to organisms that feed on carrion
Neuston	Organisms (usually zooplankton) associated with the surface film of a body of water (Thorp, 2015; Wiebe & Benfield, 2001).
Oceanography	The study of the ocean
Olfaction	Sense of smell
Oviposition	To lay eggs
Pelagic	Relating to the water column of the open ocean
Peri-mortem	At or near the time of death
Phycology	The study of algae
Poikilothermic	Organisms for whom body temperature and development is primarily governed by environmental temperature (Ames & Turner, 2003; Warzecha et al., 1999))
Post-mortem	After death
Post-mortem interval	Amount of time elapsed between death and discovery of the body
Post-mortem submersion interval	Amount of time a body has spent in water
Saponification	In this context, development of adipocere

Taphonomy	The study of how organisms decompose (and eventually become fossilised, although this is not relevant in a forensic context)
Vitellogenesis	The phase of egg growth involving accumulation of protein and lipid yolk (Engelmann, 1980)